Neurohumoral Features of Afferent Fibers in Man

Their Role in Vasodilatation, Inflammation, and Pain

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Introduction

Shortly after human skin is injured the area for several centimeters surrounding the site of injury gradually becomes reddened. This flare is a component of the inflammatory response, represents neurogenic vasodilatation,[1-3] and probably is mediated through the release of one or more vasodilator substances.[4-6] The threshold for pain in the flare zone is lowered.[7]

These phenomena have been the topic of a series of investigations in this laboratory[7-11]; in this report an attempt is made to define more carefully *(a)* the nervous pathways implicated and *(b)* the nature of the mediator substance.

Antidromic Vasodilatation after Stimulation of *the Distal Portion of Transected Dorsal Roots.*—*In 1874* Goltz[12] demonstrated that the sciatic nerve contains fibers that induce vasodilatation when stimulated. Shortly thereafter, Stricker[13] concluded that in opposition to the Bell-Magendie law, certain vasodilator fibers apparently leave the spinal cord through the dorsal roots, since he observed that stimulation of the distal portion of a transected dorsal root resulted in cutaneous vasodilatation within the segment that had been served by the root.

Bayliss,[14-17] recording the volume of the hind legs of dogs with a plethysmograph, confirmed and extended Stricker's less well-controlled observations. He ruled out the possibility that the fibers responsible for the phenomenon were spinal efferent nerves since stimulation of the peripheral portion of transected dorsal roots continued to result in vasodilatation 14 days after transection between the dorsal root ganglion and the cord. Also, he showed that extirpation of the sympathetic nervous system did not abolish the vasodilator response. In contrast, no vasodilatation resulted if the peripheral end of a transected sciatic nerve was stimulated a sufficient number of days after extirpation of the dorsal root ganglia to allow degeneration of the peripheral afferent fibers. He therefore concluded that vasodilatation resulting from stimulation of the peripheral portion of a transected dorsal root or sciatic nerve is the consequence of "antidromic" impulses passing through afferent fibers, which have their cell bodies in the dorsal root ganglia. He further showed that stimulation of dorsal roots also can result in antidromic vasodilatation in the viscera.[17] Langley also studied antidromic vasodilatation in the early part of the 20th century.[18-20] He demonstrated in cats that stimulation of a dorsal root causes flushing in the skin of the fwt. Langley postulated that this antidromic vasodilatation probably was mediated by metabolites. Hinsey and Gasser[21] and Gasser[22] further defined the fibers responsible for antidromic

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vasodilatation by showing that dorsal root stimulation must be strong enough to evoke a *C* wave in the neurogram to result in vasodilatation.

A Mediator Substance and *Antidromic* Vasodilatation.—The possibility that antidromic activity in afferent nerves results in the peripheral release of a vasodilator substance was discussed by Langley [20] and by Bayliss,[17] who both noted that there was a significant delay (2 to 8 seconds) between stimulation and the onset of antidromic vasodilatation, and that the vasodilatation long outlasted the period of stimulation. Gaskell in 1916 [23] made the first suggestion of the possible nature of the mediator substance by postulating that "acid metabolites" from skin cells were released. Doupe [24] altered this formulation to suggest that the vasodilator "metabolites" had their origin in nerve fibers, rather than in skin cells. Kibjakow [25] observed enhanced vasodilator activity in blood collected from a region of antidromic vasodilatation induced by stimulation of dorsal roots. He noted that the substance was relatively stable in blood, and thus probably was not acetylcholine. Foerster [26-27] demonstrated in man that if an adjacent root on either side of a transected dorsal root were intact, stimulation of the peripheral portion of the transected root resulted in sensations of pain, suggesting that a substance capable of stimulating pain fibers or endings is released at the terminal branches of the afferent fibers during stimulation of the root.

"H Substance" **as** *a* Mediator *for* Anti*dromic Vasodilatation.*—Lewis,[4] in an extensive series of observations with human subjects, and Krogh,[28,29] studying laboratory animals, both implicated histamine or a histamine-like substance ("H substance" of Lewis) as the agent responsible for antidromic vasodilatation. Lewis pointed out that stimulation of dorsal roots produces changes in the skin that are at least partially analogous to the "triple reaction" (i.e., local reddening of the site of injury, local edema leading to the formation of a wheal, and a more diffuse "flare" surrounding the site in the skin) that can be produced by intradermal injection of histamine. Ungar [30-32] supported the hypothesis that a histamine-like substance is released by showing that stimulation of the peripheral portion of the transected internal saphenous nerve results in increased gastric secretion similar to that produced by subcutaneous injection of histamine. The humoral nature of the phenomenon was demonstrated by showing that increased gastric secretion did not occur after stimulation of the nerve if the circulation to the limb subserved by it were *oc*cluded. However, Ungar and Parrot [33,34] concluded that the substance released into venous perfusate by stimulation of the peripheral portion of transected dorsal roots was not identical with histamine. They noted that its properties suggested that it might be an epinephrine derivative. Foerster [27] in man, and Hara ·· in the cat showed that the sweating response to stimulation of the sympathetic nervous system is inhibited in a region of antidromic vasodilatation and demonstrated that injection of histamine results in the same inhibition. Perfusate from the leg of cats, after stimulation of the peripheral portion of the transected sciatic nerve serving the leg, was observed by Kwiatkowski [36] to contain increased amounts of an agent with some of the pharmacodynamic properties of histamine. Ibrahim,[37] in support of Kwiatkowski,[36] observed that the velocity of blood flow in the skin of dogs increased after dorsal root stimulation, and that this increase was significantly reduced after injection of antihistamine agents. These observations are difficult to assess since the amount of antihistaminics used greatly lowered the blood pressure.

Thus, while there is evidence that a substance with some of the properties of histamine is released into the circulation during electrical stimulation of the distal portion of transected afferent fibers, because of the limited specificity of the bioassay methods it has not been possible to identify

the substance as histamine itself. The available evidence, while not conclusive, suggests that it probably is some other agent that appears in the blood.

It is possible to rule out histamine as the principal agent responsible for antidromic vasodilation, per se, with more confidence. Parrot and Lefebvre[38] in 1943 demonstrated that antihistamine agents do not block vasodilatation induced by stimulation of the peripheral portion of transected dorsal roots of dogs. Holton and Perry[39] showed that antihistaminics do not block vasodilatation stemming from stimulation of the peripheral portion of the transected auricular nerve in the rabbit ear. The agent they used, pyrilamine maleate, passes readily from the blood into the skin and, while it does not antagonize all the actions of histamine, it does antagonize the vasodilator effect in the rabbit ear. They observed that pyrilamine in amounts that blocked the vasodilator response from injected histamine failed to antagonize antidromic vasodilatation. Frumin, Ngai, and Wang[40] also were unable to antagonize antidromic vasodilatation with antihistamine agents.

The Concept of *"Histaminergic" Nerves.*—Ungar[41] postulated that it is a histamine-like substance that is released from the peripheral ends of afferent fibers during antidromic activity. Further evidence for the implication of histamine as a neurohumor came from the studies of Euler[42,43] and Werle,[44,45] who assayed the distribution of histamine in various regions of the nervous system. On the basis of these studies Euler[42] suggested the existence of a histaminergic system linked anatomically with the sympathetic division. Euler and Åström, studying segments of isolated peripheral nerve,[46] also reported that under certain conditions a histamine-like substance was released from one end of the segment following electrical stimulation of the other. The inferences that can be drawn from this observation as to a possible physiological role for histamine are limited by the "unphysiological" trauma that is an unavoidable feature of the technique.

Subsequent investigations have not supported the postulate that histamine is released from nerves during physiological activity and the concept of histaminergic nerves now is probably only of historical interest. The functional significance of the histamine present in the nervous system remains obscure.

Acetylcholine as a Mediator for Antidromic Vasodilatation.— Dale,[47,48] in contrast, favored acetylcholine as the mediator for antidromic vasodilatation. As early as 1863, Phillipeaux and Vulpian[49,50] had observed that stimulation of afferent nerves can result in contraction of striated muscles in the region of the endings of the afferent fibers (Vulpian-Heidenhain-Sherrington phenomenon). Since such stimulation results both in the contraction of striated muscle and in antidromic vasodilatation, Dale argued that acetylcholine probably was the mediator, for it was the only known substance to possess both actions. Later it was shown[51,52] that the contraction of striated muscle resulted from simultaneous stimulation of adjacent sympathetic fibers. Thus, the evidence concerning striated muscle is not relevant to antidromic vasodilatation induced by stimulation of afferent fibers.

Apparent experimental support for the release of acetylcholine during antidromic vasodilatation was given in the 1930's.[53-55] Wybauw[54,55] showed that arteriovenous perfusate from the posterior limb of the cat contained increased amounts of a substance with pharmacodynamic properties resembling those of acetylcholine when the dorsal roots serving the limb were stimulated. This substance resulted in a contraction of eserinized leech muscle, depression of the frog heart, and depression of the blood pressure of the cat. The origin of the acetylcholine-like substance Wybauw observed, however, is in doubt. Since Hinsey and Cutting[51] showed that the Sherrington-Vulpian phenomenon was dependent upon activation of sympathetic fibers, it is possi-

ble that the agent observed by Wybauw also stemmed from inadvertent stimulation of sympathetic cholinergic fibers. Although there is no evidence to deny that acetylcholine in small amounts may be released peripherally during stimulation of the distal portion of transected dorsal roots, whether from afferent or from sympathetic fibers, it is now considered unlikely that acetylcholine itself is responsible for the resulting vasodilatation, since antidromic vasodilatation is neither inhibited by atropine [39,56] nor potentiated by eserine.[39]

A *Vasodilator* Substance Released During *Stimulation* of *the Chorda Tympani.*—Stimulation of the chorda tympani also results in vasodilatation that is not antagonized by atropine. In 1858 Claude Bernard [57] observed that stimulation of this nerve produced salivary secretion, increased blood flow, and increased heat production. He suggested that each was produced by different types sf nerve fibers. This concept was supported in 1872 by Heidenhain,[58] who observed that the vasodilator component of the response was not antagonized by amounts of atropine that prevented salivary secretion. Thus, the enhanced salivary secretion under these conditions appears to be dependent upon a cholinergic mediator, since it is inhibited by atropine; in contrast, the mediator of the vasodilator response probably is not cholinergic, since the vasodilatation is not inhibited by atropine.

Babkin, Gibbs, and Wolff [59] showed that electrical stimulatioa of the chorda tympani resulted in a lowering of the arterial blood pressure. The depressor response could not be observed unless the animal was treated with an anticholinesterase (Physostigmine), indicating that an acetylcholine-like substance was implicated in the release of a depressor substance into the blood during stimulation of the chorda tympani. On the other hand, Ungar et al.[60] demonstrated that stimulation of the peripheral portion of the transected lingual nerve in the atropinized dog resulted in heightened gastric secretion, thus also implicating a histamine-like substance, presumably carried to the stomach by the circulating blood.

Feldberg and Guimarais [61] showed that saliva contains a substance that causes a fall in blood pressure when injected intravenously. It could be differentiated from both histamine and acetylcholine. Ungar and Parrot [62] identified this hypotensive agent in saliva as kallikrein,[63-66] a proteolytic enzyme that forms a vasodilator polypeptide when incubated with plasma globulin. It has been difficult to separate the actions of kallikrein, per se, from those of the polypeptide formed when the enzyme acts on globulin, but presumably the substance responsible for the fall in blood pressure is kallidin, a polypeptide formed by kallikrein acting on globulin present in the blood of the animal used for bioassay. Ungar and Parrot also observed that kallikrein stimulates gastric secretion and thus might possibly be the histamine-like substance that they had observed after stimulation of dorsal roots. They suggested that kallikrein can sometimes "play the role of a neurshumoral mediator." [62] Szakall [67] postulated that the "H substance" of Lewis is identical with kallikrein.

Recently Hilton and Lewis [68-70] showed that a substance (presumably a proteolytic enzyme) capable of forming a vasodilator polypeptide when incubated with plasma globulin, is present in increased amounts in perfusate of the salivary gland after stimulation of the chorda tympani. They did not believe that the enzyme stemmed from nerve cells, but concluded that acetylcholine released by the postganglionic nerve fibers acts on the gland to heighten its cellular metabolism, and that it is this heightened metabolism that leads to a release of a proteolytic enzyme from the cells sf the salivary gland. A similar substance has been observed in subcutaneous perfusate of the skin during thermoregulatocy sweating [71] and in perfusate of the cat's tongue during stimulation of the chordolingual nerve.[72] Hilton and Lewis [72] failed to observe an increase of prsteolytic enzyme content of

arteriovenous perfusate of the skin of the cat during stimulation of the distal portion of the transected saphenous nerve.

Substance P as a Mediator of Antidromic Vasodilatation.—Hellauer and Umrath [73] observed that extracts of dorsal roots possess greater vasodilator activity than do extracts of ventral roots. The activity of the extracts declined with time, but could be preserved by strychnine. They postulated that this vasodilator substance acted as a synaptic transmitter within the central nervous system. Lembeck [74] concluded that the vasodilator substance in dorsal roots is substance P, and that it is a mediator of antidromic vasodilatation. Substance P[75-78] is a vasodilator polypeptide that has been extracted from intestinal mucosa [75,79] and from brain tissue,[75,80-82] as well as from dorsal roots.[79-81] To the present time there is no evidence that this substance is released during stimulation of dorsal root fibers. However, some evidence in favor of the hypothesis that substance P is implicated has been presented. By analogy with acetylcholine, Holton [83,84] reasoned that the "transmitter substance" of afferent nerves would disappear rapidly from a mixed nerve during the degeneration of afferent fibers. Accordingly, segments of peripheral nerve were analyzed for substance P and for adenosine triphosphate (ATP). After transectioa the content of substance P fell rapidly, while that of ATP did not. Sympathectomy did not alter the content of either substance.

The evidence implicating substance P in antidromic vasodilatation is inconclusive. While it does occur in the nervous system and is present in particularly large amounts in the dorsal roots, there is no evidence that it is released during antidromic vasodilatation.

ATY as a *Mediator* of *Antidromic Vaso*-dilatation.—Holton and Holton [85] demonstrated that ATP is present in dorsal root extracts. Recently Holton has shown that ATP is released into arteriovenous perfusate of rabbits' ears during and immediately following stimulation of the peripheral portion of the transected great auricular nerve.[86] The bioassay method (firefly luminescence) apparently is highly specific for ATP.[87] The degree to which ATP contributes to antidromic vasodilation remains to be determined since the amounts of ATP that could be recovered in the perfusate were small. The amount of ATP required to induce a degree of vasodilatation equivalent to that induced by a given stimulation was 100,000 times more than the maximum amount of ATP that could be recovered in the effluent. (In contrast Brown, Dale, and Feldberg [88] were able to recover one part in a hundred of the amount of acetylcholine theoretically liberated in perfusate of sympathetic ganglia.)

Neurogenic Vasodilatation (Flare) Evoked by Noxious Stimulation of Skin.—Müller in 1913 pointed out that the flare response from pinching, pricking, cutting, or other noxious stimulation is absent in regions of skin deprived of their nerve supply because of injuries to peripheral trunks. Breslauer [89] induced flare in human skin by application of mustard oil and noted that while Rare did not occur in regions of skin that had been deprived of their innervation weeks or months before, it could still be evoked in patients with recent nerve injuries.

Müller had suggested that cutaneous flare is dependent upon a spinal cord reflex, a view accepted by Krogh [29] and Ebbeke,[90] but Breslauer [89] and Lewis and Grant,[2,6] studying persons with peripheral nerve injuries, concluded that an "axon reflex" mechanism similar to that described by Bruce [91] and Bardy [92] in the conjunctiva was correct, since they showed that a flare response could be evoked for several days after the pathways to the cord had been interrupted.

The Concept of the "Axon Reflex".—Langley and Anderson [93] in 1894 suggested the possibility that certain reflexes take place entirely within a single neuron. Their formulation originally referred to the preganglionic neurons of the sympathetic

nervous system and later was extended to include reflex vasodilatation in intact skin.[18,19] Langley[20] pointed out that the same nervous elements probably are implicated in both the cutaneous vasodilatation of the flare response to noxious stimulation and in the antidromic vasodilatation produced by stimulation of dorsal roots.

Woollard[94] furnished anatomic evidence to support the concept of axon reflex vasodilatation by showing extensive branching of afferent nerves at their terminations and the proximity of some of the branches to the walls of the blood vessels. Recently Weddell[95-96] has carried out additional studies of morphological features of cutaneous nerves. Physiological evidence for the existence of axon reflexes in the skin of frogs in which evoked responses to stimuli were observed both locally and several centimeters from the site of stimulation was presented by Adrian, Cattell and Hoagland.[97]

Ebbeke,[90] Krogh,[29] Bruce,[91] and Lewis[4] extensively explored the participation of the nervous system in local vascular reactions. Bruce[91] showed that vasodilatation and other features of inflammation after noxious stimulation of the conjunctiva of the rabbit are suppressed by local anesthetics and are diminished after degeneration of afferent fibers resulting from extirpation of the trigeminal ganglia serving the region. Similarly, Krogh[29] observed that capillary dilatation following application of noxious agents to the tongues of frogs disappeared if the nerve fibers were rendered inoperable by topical application of local anesthetics. Lewis and Grant's observations[2,6] demonstrated in subjects with peripheral nerve injury that the flare component of the triple response of the human skin to noxious stimulation could be evoked for 6 or 7 days after section of the nerve and then was absent. Thereafter, only the wheal and the "central red area" directly beneath the region stimulated noxiously could be observed. While they established that the link to the central nervous system is not essential to the phenomenon of flare, they did not eliminate the possibility that, in the intact person, reflexes involving the central nervous system also contribute. Reasoning from the dorsal root ganglionectomy experiments of Bruce[1] and of Bayliss,[17] these authors postulated that it is the degeneration of the afferent fibers in the skin that leads to absence of the flare. However, since the subjects studied had undergone transection of mixed nerve, the regions they studied lacked efferent and autonomic innervation as well.

With laboratory animals, Celander and Folkow,[98,99] by means of electrophysiological recording techniques, studied the fibers that participate in vasodilatation evoked by noxious stimulation and concluded that it is only "pain" fibers that are implicated, and that the fibers mediating temperature sensation do not contribute; i.e., "the function of the axon reflex is solely to evoke a local increase of the blood flow through superficial tissues exposed to noxious influences." They also noted that even very slow rates of discharge in the fibers that respond to noxious stimulation may induce almost maximal vasodilatation.

Parrot[100] in 1943 suggested that the fibers responsible for cutaneous flare evoked by noxious stimulation are "not sensory but centrifugal fibers, just as in all other types of axon reflexes."[101] Kure[102] also suggested that the fibers responsible for the vasodilatation resulting from stimulation of the peripheral portion of transected dorsal roots were efferent fibers. However, to the present time, the existence of efferent fibers in the dorsal roots has not been demonstrable. The conclusion that all of the nerve fibers having their cell bodies in the dorsal root ganglia are afferent fibers is supported by the evidence that no synapses have been found in the spinal ganglia, and by the observation that the ratio of the total number of fibers, myelinated and unmyelinated in a root between the ganglion and the cord to the total number of cells, large and small, in the spinal ganglion is 1:1.[103]

The Nature of the Mediator Substance *Implicated in* Axon Reflex Flare.—Lewis[4] in 1927 postulated that noxious stimulation of the skin resulted in the local release of a histamine-like agent from cells in the skin. This agent, he suggested, stimulated the dermal endings of branched afferent fibers in the region, and through axon reflexes produced dilatation of small vessels. Krogh[29] extended this model by postulating that "H substance" is released at the terminations of the afferent fibers activated in these axon reflexes, and thus serves as the vasodilator mediator between the neuron and the blood vessels.

Dale and Gaddum[48] favored acetyicholine as the agent acting between the neuron and the blood vessel. However, since it is now established that antidromic vasodilatation evoked by stimulation of the distal portion of transected afferent fibers is neither inhibited by atropine nor potentiated by eserine,[39] it is unlikely that acetylcholine is the vasodilator substance active at this site in axon reflex vasodilatation.

Lewis' concept[4] that histamine released locally in skin exposed to noxious stimulation can serve to activate afferent nerves in that region has been supported by subsequent findings,[104] but the suggestion that histamine is released at the termination of the activated neurons has not.

Antihistaminics diminish the cutaneous flare induced by intradermal administration of histamine or snake venom and the flare reaction to cold in patients with urticaria.[104] In contrast, flare evoked by electrical stimulation of human skin, presumably stimulating cutaneous nerves directly, is not antagonized.[104] These observations suggest that histamine is implicated in reflex vasodilatation only within the zone of noxious stimulation, and not at the termination of the activated neurons. Parrot[101] concluded that "after irritation of the skin, histamine released by injured cells is able to stimulate peripheral endings of the vasodilator fibers and elicit a vasodilator response," and, therefore, "histamine acts not as a mediator at the end of the axon reflex, but as the stimulus at its origin."

Observations from this laboratory[105] apparently indicated that histamine was implicated in the vasodilatation occurring near the termination of fibers activated in local neuron reflexes, since it was demonstrated that an antihistamine agent, tripelennamine, dropped onto the surface of the eye antagonizes the vasodilatation of the bulbar conjunctiva that results from noxious stimulation (application of hypertonic saline) to the nasal mucosa. However, the significance of this observation for the role of histamine, per se, in neurogenic vasodilatation is limited by the possibility that in relatively high concentration, such as might be achieved during local application in the eye, antihistiminics may antagonize the action of substances other than histamine. Indeed, tripelennamine (1-5 mg/kg) and other antihistamine agents have been shown in this laboratory by Zileli to antagonize the hypotensive action of certain vasodilator polypeptides.[106]

Does the Mediator of *Antidromic* Vasodilatation Lower the *Threshold* for Neuron *Firing?*—Foerster's experiments,[27] in which painful sensations were induced by stimulation of the peripheral portion of transected dorsal root fibers, and the observations of Bilisoly et al.[7] that the pain threshold is lowered during the phase of active vasodilatation in regions of cutaneous flare induced by noxious stimulation, raise the possibility that the neurohumoral vasodilator mediator substance has the ability to lower the threshold for activating the neurons subserving pain- sensation (although it is conceivable that these actions are produced by separate mediators). Experimental evidence that a substance released during stimulation of peripheral ends of transected cutaneous nerves has the action of lowering the threshold for firing of afferent nerves has been furnished recently by Habgood.[107] He removed 2 pieces of frog skin, preserving a length of cutaneous nerve in each. The 2 pieces were placed in such a way that

their under-surfaces were contiguous. He recorded from the nerve of one and stimulated the nerve serving the other. He demonstrated that the threshold for evoking a response in the nerve of the first piece of skin by applying pressure on it was lowered when the nerve serving the other piece of skin was stimulated. At times, stimulation of one nerve would lead to spontaneous activity in the other. Then, studying single pieces of skin with several cutaneous nerves preserved in such a way that the areas subserved by 2 of them overlapped, he showed that stimulation of one of these nerves led to spontaneous responses in the adjoining nerve. He inferred that a neurohumoral agent, perhaps similar to histamine, was released by stimulation of the first nerve that lowered the threshold of discharge for the second.

Thus, much evidence indicates that when, as the result of noxious stimulation, impulses pass antidromically in the terminal branches of certain peripheral nerves having their cell bodies in the dorsal roots a neurohumoral substance is released or formed. In an attempt to define this substance, previous studies from this laboratory[8,10] established that in man the amount of a substance with pharmacodynamic properties resembling those of the group of vasodilator polypeptides called kallidin,[66] bradykinin,[108] or plasma kinin[109] increases significantly in subcutaneous perfusate during the period of active vasodilatation of flare induced by noxious stimulation. It was also observed[110,111] that a substance with similar properties * is formed when cerebrospinal fluid (CSF) collected from patients with disorders of the central nervous system is incubated with plasma globulin. (See Table 1.)

Experiments were conducted to extend these observations and to define the properties of the relevant agent more precisely.

Method

1. The Neuroanatomic Basis of Cutaneous Vasodilatation (Flare) Evoked by Noxious Stimulation of the Skin.—The cutaneous response to intradermal injection of histamine (0.05 *cc.* histamine phosphate 1:1,000) was observed in subjects with *(a)* transection of peripheral nerve, *(b)* sympathectomy, *(c)* section of dorsal root between the ganglion and the spinal cord, and (d) extirpation of dorsal root ganglia. The color and the extent of the wheal and, when present, the flare, were recorded.

2. The Neurohumoral Basis of Cutaneous Vasodilatation (Flare) Induced by Noxious Stimulation of the Skin.—*A.* Flare Evoked by Noxious

* Also, in laboratory animals, the capacity of perfusate of the cerebral ventricles and subarachnoid space to form this substance when incubated with globulin was increased after stimulation of the central portion of the transected sciatic nerve, suggesting that a proteolytic enzyme was released within the brain.[112]

TABLE 1.—Cerebrospinal Fluid Incubated with Plasma Globulin: Number of Milliunits of Bradykinin Required to Induce a Response Equal to That Induced by 1 Cc. CSF

Specimen No.	Duodenum	Uterus	Blood Pressure	U./D.	B.P./D.
CSF 1	40	2.2	2,000	0.055	50
2	50	2.6	2,000	0.053	40
3	60	--	2,000	--	33
4	35	--	800	--	23
5	25	1.25	--	0.050	
			Average	0.052	36

The specimens of cerebrospinal fluid were collected from hospitalized patients with a diagnosis of chronic schizophrenia. The cerebrospinal fluid was collected into polyethylene tubing and stored in solid carbon dioxide. Immediately after thawing specimens of cerebrospinal fluid were incubated with an equal volume of a 1% solution of globulin (Nutritional Biochemical Company, Bovine Globulin Fraction II) for 3 minutes at 37 C and then placed in a boiling water bath for 5 minutes. After cooling, the specimens were divided for assay into 2 portions. One portion was added to a smooth muscle bioassay chamber containing both rat uterus and rat duodenum, and the other was injected intravenously into a rat prepared for recording of arterial blood pressure.

The response of the uterus and the duodenum, as well as the blood pressure, to several amounts of bradykinin had previously been determined. The response to the specimen of incubated CSF + globulin then was expressed as the amount of bradykinin required to induce an equivalent response. The relatively constant ratios of uterus to duodenum and blood pressure to duodenum suggest that the observed activity stems from a single substance.

Stimulation of the Skin: The subcutaneous tissue of the volar surface of the forearm was perfused with isotonic saline and the perfusate assayed before and after flare was induced by pinching and crushing with forceps, by faradic stimulation of the skin, by thermal injury with radiant energy, or by intradermal injection of histamine (0.05 cc., 1:1,000). Noxious stimulation of the skin was applied 2 to 4 cm. distant from the location of the perfusion needles to insure that the perfused region was in the zone of "flare," and not directly underlying the damaged area. Forty-three perfusion experiments on 12 subjects were performed.

B. Reactive Hyperemia: By way of contrast, non-neurogenic vasodilation (reactive hyperemia) was induced after 7 to 12 minutes' interruption of the circulation of the arm by means of a manometer cuff about the upper arm in 4 normotensive subjects. The cuff was inflated to a pressure of 220 mm. of mercury. Samples of subcutaneous perfusate were collected during a 6-minute control period before the cuff was inflated, during the period of occlusion, and for 5 3-minute intervals during the period of reactive hyperemia after deflaticm of the cuff.

C. Cutaneous Vasodilatation Induced by Stimulation of Dorsal Roots: The subcutaneous tissue underlying the skin subserved by dorsal nerve roots being sectioned for relief of intractable pain in patients with tumor of the apex of the lung was perfused with isotonic saline. The perfusate was assayed before and after antidromic vasodilatation was induced by mechanical (surgical trauma) and electrical † stimulation of the peripheral portion of the divided dorsal root. A series of experiments with 3 subjects was performed.

D. Collection of Subcutaneous Perfusate: The method used was a modification of that of Fox and Hilton.[71] Two hypodermic needles (No. 20) with 4 perforations in the shafts (specially made by Becton, Dickinson & Co., Rutherford, N.J.) were inserted into the relevant subcutaneous tissue parallel to each other and approximately 1.5 cm. apart. Sterile isotonic saline (0.05 cc/min.) was allowed to drip into the tissues through one needle. The outflow from the other needle was collected in a siliconed syringe (1 cc. or 0.25 cc.) or in a 20-inch length of polyethylene tubing (Venotube, Abbot Laboratories) attached to the outflow needle. Slight negative pressure was maintained by means of a 50 cc. syringe joined to the other end of the tubing. The perfusion site was gently massaged to facilitate flow. Specimens were assayed immediately after the collection period or stored in solid carbon dioxide until assayed. In order to "pool" samples collected over periods longer than 3 minutes, the samples were stored in 1 cc. polyethylene centrifuge tubes suspended in ice water for 10-30 minutes until assayed.

E. Reinjection of Perfusate: In 3 subjects, perfusate collected before and during flare induced by pinching and crushing the skin with forceps 4 cm. from the perfusion needles was reinjected into the skin of the back. After insertion of the needles and introduction of approximately 2 cc. of sterile saline, the needles were left in place for 30 minutes to allow the reaction of the perfusion procedure to abate. Perfusate was then collected during 5-minute intervals into a small (0.25 cc.) siliconed syringe and aliquots (0.05 cc.) of the perfusate collected during that time reinjected intradermally with a No. 25 needle. The subject was queried about the nature of the sensations that resulted from the injections. The site of the injections was observed carefully for 10 minutes and the maximal extent of the wheal and the flare were outlined with ink. The outlines were then transferred to tracing paper.

F. Bioassay with Smooth Muscle: Virgin female rats (150-200 gm.) were stunned by a blow on the head and bled to death. The bicornuate uterus and the first 8 to 10 cm. of duodenum proximate to the pylorus was immediately removed and placed in modified Ringer-Locke solution. In most assay procedures this solution contained per liter, NaCl, 9.0 gm., KCI, 0.42 gm., $CaCl_2$, 0.06 gm., dextrose, 0.5 gm., $NaHCO_3$, 0.5 gm., and distilled water to volume. In others, the $CaCl_2$ was reduced to 0.02 gm/L.

A single horn of the uterus and a segment of duodenum were cut to suitable length (4-5 cm.) and the adherent connective tissue, blood vessels, and fat carefully dissected away. After rinsing the interior of the tubular muscles with the saline solution they were tied at both ends with cotton thread. Both muscles were mounted in a single 3 cc. chamber, one end of each being secured to the bottom of the chamber and the other to a frontal recording lever that recorded the relaxation or contraction of the muscles on a smoked paper kymograph. The chamber was filled with the saline solution and a constant vigorous stream of room air introduced in the bottom of the chamber through a small (No. 25) hypodermic needle. The entire chamber was placed in a constant temperature water bath maintained at 29 C. Since preliminary studies[8,10] had demonstrated that a substance resembling bradykinin[108] appeared in subcutaneous perfusate during reflex flare induced by noxious stimulation, this substance was used to calibrate the bioassay systems. (Bradykinin was kindly furnished by Professor Maurici Rocha e Silva, University of São Paulo, Ribeirão Prêto, Brazil.) After mounting the muscles in the chamber, the leverage and counterbalance weights were adjusted until responses of suitable

† 60 c.p.s. sine wave, 3 volts, 2 minutes, Hinsey-Geohagan stimulator.

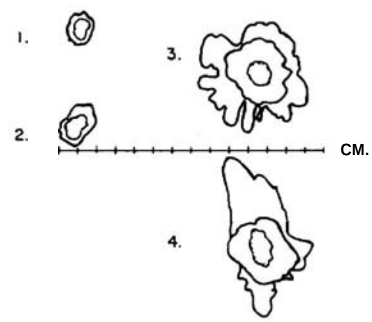
magnitude to *2-5* milliunits of bradykinin were achieved (magnification approximately 18 times for duodenum, 10 times for uterus; appropriate tension was induced by weights of approximately 0.5 gm. for uterus and 0.25 gm. for duodenum). If an adequate response to 5 milliunits of bradykinin could not be achieved the muscles were discarded. The responses to several amounts of bradykinin in the range anticipated to be appropriate for the specimens were then recorded. **At** least 3 different points for a "dose response curve" were plotted. Aliquots of the specimens were then added each 6 minutes and allowed to remain in the bath for 1½ minutes. The muscles were washed by alternate filling and draining of the chamber 3 times with the saline solution. For assay with the guinea pig ileum, Tyrode solution maintained at 37 C was used.

G. Bioassay with Rat Blood Pressure: Rats weighing 150-200 gm. were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg and additional amounts as necessary to maintain anesthesia). After tracheotomy, small bore polyethylene tubing was introduced into the jugular vein or the dorsal vein of the penis to permit intravenous administration of the material to be assayed. Polyethylene tubing joined the carotid artery to a recording manometer for registration of arterial blood pressure. As an anticoagulant, 50 units of heparin in 1 cc. of isotonic sodium chloride solution were injected intravenously. The depressor response to 500 and to 1,000 milliunits of bradykinin served to calibrate the respcmse to specimens of the subcutaneous perfusate. The blood pressure was allowed to recover to the original pressure between administration of specimens.

Results

I. Properties of Subcutaneous Perfusate Collected During Vasodilatation Evoked by Noxious Stimulation of the Skin. — *A.* Reinjection of perfusate: Perfusate collected during the control period, (30 minutes or more after inserting the needles and beginning the perfusion) resulted in pain no greater than that resulting from injection of an equal volume of isotonic saline. The wheals and the flare evoked by control perfusate also approximated in size and color those resulting from isotonic saline. When flare was induced by pinching and crushing of the skin adjacent to the perfusion area, the perfusate acquired the capacity to induce more severe pain than that induced by intradermal injection of isotonic saline. The

TRACINGS OF FLARES
INDUCED BY RE-INJECTION OF PERFUSATES



CONTROL PERFUSATE

PERFUSATE COLLECTED DURING NOXIOUS STIMULATION

Fig. 1.—Tracings of area of wheal and of flare reaction induced by intradermal reinjection of subcutaneous perfusate, demonstrating that a mediator substance is elaborated during the flare reaction. Perfusion needles were inserted into the subcutaneous tissues of the volar surface of the forearm and slowly perfused with normal saline (1 drop introduced each 20 seconds). A 30-minute period was allowed to elapse to permit the reaction to the injury of establishing the perfusion procedure to diminish. The perfusion rate was then raised to 12 drops per minute. Perfusate was collected directly from the perfusion needles into a siliconed syringe. After a 5-minute collection interval, an aliquot (0.05 cc.) of the perfusate was reinjected intradermally into the skin of the subject's own back. The maximum extent of the peripheries of the wheal and the flare were outlined in ink and traced onto transparent paper. (1) and (2)—Reactions to control perfusate. The skin 4 cm. from the perfusion needles was then pinched and crushed with forceps to induce a flare reaction. (3)—Reaction to perfusate collected during first 5-minute interval after beginning noxious stimulation. (4)—.Reaction to perfusate collected during second 5-minute interval after beginning noxious stimulation.

pain was "burning" in quality, and lasted for 15 to 30 seconds after the injection. The area of the flare induced by this latter perfusate was predictably greater than that induced by control perfusate, and the flare zone had a more intense red color (see Fig. 1).

B. Bioassay of the Perfusate: The initial specimens of perfusate collected shortly after inserting the needles contracted the uterus and relaxed the rat duodenum, but were inert as regards the blood pressure of the rat. The activity of subsequent specimens as assayed with the uterus and duo-

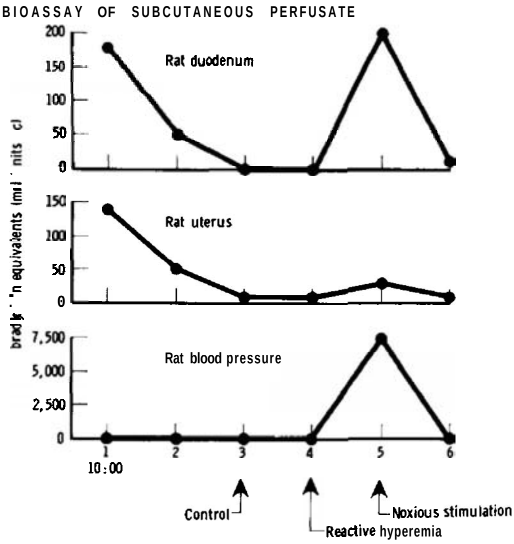


Fig. 2.—Bioassay of subcutaneous perfusate, demonstrating that a pharmacodynamically active material is elaborated during flare evoked by noxious stimulation, but not during reactive hyperemia. The 3 bioassay systems were calibrated with bradykinin. (1) Perfusate collected during the first 5 minutes after inserting the perfusion needles. (2) Six to 12 minutes after insertion of needles. (3) Twelve to 18 minutes after needle insertion (control level). (4) During reactive hyperemia (0 to 6 minutes after deflating occlusion cuff). (5) Zero to 6 minutes after beginning noxious stimulation of the skin (faradic current for 3 minutes) and during onset of reflex flare. (6) Six to 12 minutes after beginning noxious stimulation of the skin. The specimens of perfusate were pooled at 3-minute intervals and stored at 3-4 C.

denum diminished to a low constant level approximately 10-20 minutes after beginning the perfusion. This base line level of activity sometimes was too small to be observed. The perfusate collected during the onset of flare predictably increased in its ability to relax the isolated rat duodenum and to contract the rat uterus. It also acquired the capacity to depress the blood pressure of the rat (0.05 cc. of the active perfusate resulted in a fall of blood pressure equivalent to 30-50 mm. of mercury, Figs. 2 and 3 and Table 2). The capacity of the perfusate to induce contractions of the guinea pig ileum did not increase above a low control level during the course of the flare reaction.

During the onset of flare, although the input rate remained constant, the volume of fluid collected from the outflow needle increased from 3 to 9-fold during the first 6-9 minutes after noxious stimulation. The swollen region at the perfusion site also became softer and its boundaries less well defined.

Specimens lost their activity when allowed to stand at room temperature for 15 to 30 minutes, but they could be stabilized by heating in a boiling water bath for 5 minutes. Incubation with chymotrypsin for 5 minutes at 37 C inactivated the stabilized specimens, suggesting that the active substance is a polypeptide, possibly produced by proteolytic enzyme action (Fig. 4). Evidence that in addition to the polypeptide, the perfusate collected immediately after noxious stimulation also contains a proteolytic enzyme capable of forming the polypeptide was obtained through the observation that perfusate collected at this

TABLE 2.—*Subcutaneous Perfusate Collected from a Region of Reflex Vasodilatation Evoked by Noxious Stimulation of the Skin: Number of Milliunits of Bradykinin Required to Induce a Response Equal to That Induced by 1 Cc. of Specimen*

Specimen No.	Duodenum	Uterus	Blood Pressure	U./D.	B.P./D.
Reflex flare 1 perfusate	600	30	20,000	0.050	23
2	50	--	1,200	--	25
3	160	10	--	0.062	--
4	200	48	7,500	0.090	37.5
			Average	0.067	31

Specimens of subcutaneous perfusate collected during the 0-to-3-minute and the 3-to-6-minute intervals after beginning noxious stimulation (faradic current) were pooled. The container of the pooled specimens was maintained at the temperature of ice water until it was placed in a boiling water bath for 5 minutes. After cooling, the specimens were assayed as in Table 1. As with the cerebrospinal fluid-globulin mixtures, the relatively constant ratios observed suggests that the activity stems from a single subetance.

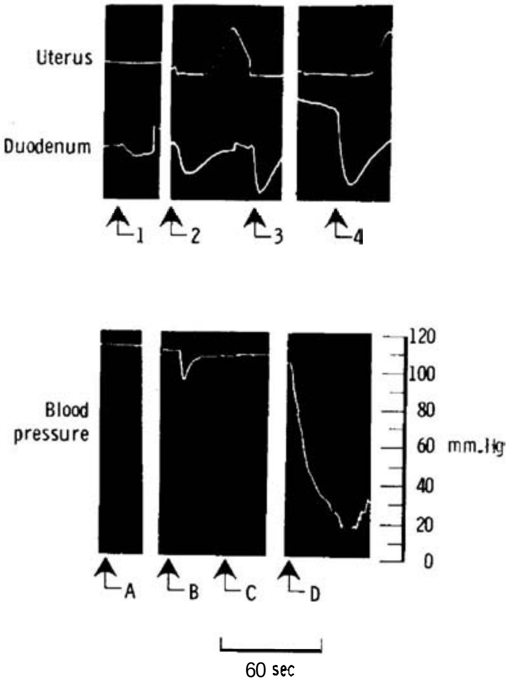


Fig. 3.—Bioassay of subcutaneous perfusate collected before and during reflex flare induced by faradic stimulation, showing the action of the perfusate on isolated rat duodenum, isolated rat uterus, and blood pressure of a rat. (1) Pooled "control" sample, 0.1 cc., collected during a 6-minute period beginning 12 minutes after insertion of the needles and ending shortly before noxious stimulation of the skin (faradic current). (2) Bradykinin, 20 milliunits. (3) Pooled perfusate, 0.1 cc., collected during the first 3 minutes of the reflex flare. (4) Pooled perfusate, 0.1 cc., collected during the 3- to 6-minute interval of reflex flare. *(A)* 0.2 cc. pooled control perfusate; (B) bradykinin, 500 milliunits; (C) 0.1 cc. pooled perfusate collected during the first 3 minutes of reflex flare; (D) 0.1 cc. of pooled perfusate collected during 3 to 6 minute interval of reflex flare. The pooled samples were stored in ice water for 10-20 minutes at 3-4 C until they were assayed.

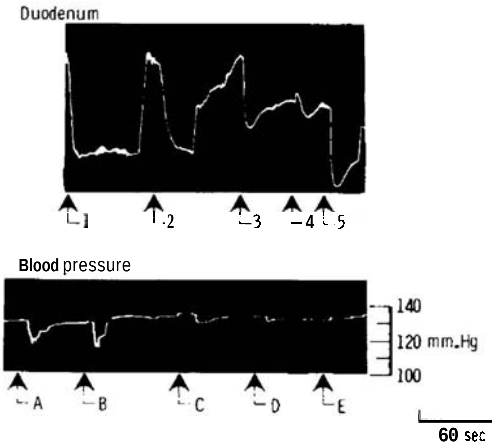


Fig. 4.—Destruction of active substance in perfusate collected during reflex flare by incubation with chymotrypsin. Reflex flare specimens were collected and pooled during the 6-minute period after the beginning of noxious stimulation (faradic current). The specimens were then stabilized by 5-minute immersion in a boiling-water bath. Chymotyrpsin (1 mg. of chymotrypsin per cc. of perfusate) was allowed to act at 37 C for 3, 5, and 7 minutes. The action of the enzyme was stopped by reheating in the boiling water bath. (1) 0.1 cc. perfusate, without chymotrypsin; (2) 0.2 cc. perfusate, 3-minute incubation with chymotrypsin; (3) 0.2 cc. perfusate, 5-minute incubation, with chymotrypsin; (4) 0.2 cc. perfusate, 7-minute incubation, with chymotrypsin; (5) 0.1 cc. perfusate without chymotrypsin. *(A)* Bradykinin, 500 milliunits; (B) 0.1 cc. perfusate, no chymotrypsin; (C) 0.2 cc. perfusate, 3-minute incubation with chymotrypsin; (D) 0.2 cc. perfusate, 5-minute incubation, with chymotrypsin; (E) 0.2 cc. perfusate, 7-minute incubation with chymotrypsin.

time increased in potency if incubated with globulin for 3 minutes (Fig. 5). Perfusate collected shortly thereafter lost potency during incubation.

The heat-stabilized specimens demonstrated a constant ratio of activity (as related to the bradykinin standard) on rat uterus, rat duodenum, and rat blood pressure among specimens from several experiments and for a single specimen assayed after 3-, 5-, and 7-minute incubation with chymotrypsin. These observations suggested that the pharmacodynamic properties observed in the perfusate during the development of flare evoked by noxious stimulation are due to a single substance. The possibility that this constant ratio resulted from a mixture with uniform proportions of different substances seems less likely (Table 2).

The substance in the heat-stabilized specimens of perfusate had many of the pharmacodynamic properties of oxytocin [113] and of the vasodilator polypeptides termed bradykinin,[114] kallidin,[115] and plasma kinin.[109] However, when analyzed quantitatively using several assay procedures, it became less likely that the substance is identical with any of these. Polypeptides were prepared by incubating the following proteolytic enzymes with plasma globulin followed by heating in a boiling water bath: plasma kallikrein, urinary kallikrein, pancreatic kallikrein, and trypsin. It was possible

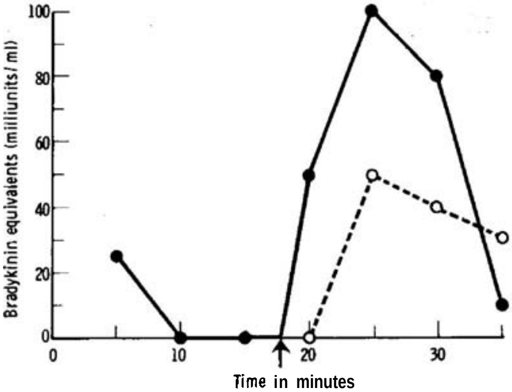


Fig. 5.—Bioassay (isolated rat duodenum) of subcutaneous perfusate collected before and after noxious stimulation (faradic current for 2 minutes) of the skin, demonstrating that perfusate collected immediately after noxious stimulation, but not thereafter, increases in its pharmacodynamic activity if incubated with globulin, indicating the presence of a proteolytic enzyme at that time. The arrow indicates the beginning of noxious stimulation of the skin. The perfusate was divided into 2 portions; one-half was assayed immediately (open figures), and the other half was incubated with globulin for **3** minutes before being assayed (solid figures).

to discriminate between the properties of the substance formed during flare from those of each of the polypeptides formed by these proteolytic enzymes. For example, reduction of the calcium concentration in the nutrient bath (from 0.06 to 0.02 gm. $CaCl_2$/L) permitted discrimination of the substance from bradykinin, the polypeptide formed by brief incubation of globulin with trypsin or snake venom, since it was found that in this solution the uterus retains its sensitivity to bradykinin but is much less sensitive to the substance observed in the perfusate. When equated in terms of the response of the duodenum, in solutions containing 0.02 gm. $CaCl_2$/L, the ratio of activity of the substance to bradykinin on the uterus was approximately 1 to 15. In solutions containing 0.06 gm. $CaCl_2$/L the ratio was approximately 1 to 2. Also, when judged equiactive by bioassay with the duodenum, the substance was much more potent than bradykinin on the rat blood pressure. When equated in terms of the response of the duodenum in solutions containing 0.02 $CaCl_2$/L, the ratio of the activity of the substance to bradykinin on the rat blood pressure was approximately 36 to 1.

The properties of the substance are not those of epinephrine, norepinephrine, hypertensin, pepsitensin, acetylcholine, histamine, serotonin, adenosine, ATP, potassium or isopropyl norepinephrine (Table 3). It has certain features in common with, but can be differentiated from, a number of vasodilator polypeptides and protmlytic enzymes that form vasodilator polypeptides. It closely resembles oxytocin, but when equated in terms of the response of the duodenum, it has a more powerful depressor action on the blood pressure of the rat and

TABLE 3.—*Differentiation of Other Substances That Occur in the Body from the Pharmacodynamically Active Substance Present in Heat-Stabilized Specimens of Subcutaneous Perfusate Collected from a Zone of Cutaneous Vasodilatation-Induced by Noxious Stimulation of the Skin*

A. The pharmacodynamic properties of the perfusate diminish slowly at room temperature but can be observed for 10-15 minutes after collection. The activity of the perfusate can be stabilized by boiling in a water bath, and the activity of heat stabilized specimens is destroyed by brief incubation with chymotrypsin. These observations indicate that the active substance is a polypeptide and permit its discrimination from a number of nongolypeptide local hormones, neurohumors, and agents that have been suggested to be relevant to pain.
   1. Agents that are not destroyed by chymotrypsin: Epinephrine, norepinephrine, isopropyl norepinephrine, acetyleholine, adenosine, potassium, histamine, and ATP
   2. Proteolytic enzymes: Kallikrein—pancreatic, urinary, or from plasma,[66,116] plasmin,[109] globulin permeability factor[142,185]

B. Since the substance depresses the arterial blood pressure of the rat it can be discriminated from polypeptides that are hypertensive or that do not alter the blood pressure of mammals:
   1. Vasopressin,[113] hypertensin, pepsitensin, pepsaneurin and pepsitocin,[187] leukotaxine.[171] Of the hypotensive polypeptides, the substance can be differentiated from those that induce contraction of the isolated rat duodenum: substance P[18]*

D. Parallel assay with the rat uterus, rat duodenum, and blood pressure of the rat permits discrimination of the substance from others qualitatively similar:
   1. In de Jalon's solution the amount of the substance necessary to induce a minimal relaxation of the isolated rat duodenum is approximately the same as the amount necessary to induce a minimal contraction of the rat uterus. In contrast, approximately 1,000 times more oxytocin is required to induce relaxation of the rat duodenum than is required to contract the rat uterus.[189]
   2. When equated in terms of the response of the rat duodenum, bradykinin is less active on the rat blood pressure and more active on the rat uterus than is the substance (see Figs, 2 and 3). Kallidin (formed by human urinary kallikrein acting on ox globulin) and pain-producing substance (formed by glass activation of human plasma) are apparently not distinguishable from bradykinin when assayed in parallel on different tissues.[190]

TABLE 4.—Subcutaneous Perfusate Collected from Regions of Noxious Stimulation of the Skin: Number of Milliunits of Bradykinin Required to Induce a Response Equal to That Induced by 1 Cc. of Specimen

Specimen No.	Duodenum	Uterus	B.P.	U./D.
Initial #1	95	100	No action	1.05
Perfusate #2	200	200	No action	1.00
#3	150	170	No action	1.13
Thermal Injury #1	40	50	No action	1.25

The initial, perfusate was collected during the 3-minute-interval immediately after introducing the perfusion needles and beginning the inflow of saline. The thermal injury specimen was collected after a suitable control period during which the activity in specimens had fallen below the limits of sensitivity of the method. The specimen was collected during the 3-minute interval after applying a damaging amount of thermal radiation (500/mc/cm sq./sec for 3 seconds) to a region of blackened skin directly over the perfusion site.

is less powerful in its capacity to contract the uterus.

The elaboration of the active substance in subcutaneous perfusate is not the result of vasodilatation alone, since in all 4 instances perfusate collected during nonneurogenic vasodilatation (reactive hyperemia) did not increase in activity above control levels with the methods used (see Fig. 2).

II. ***Bioassay of Perfusate Collected Directly beneath a Zone of Thermal Injury.***—In 2 experiments the skin directly over the perfusion site was burned by exposure to radiant energy. This procedure resulted in a small increase in activity of the perfusate on the uterus and duodenum. With the amounts of perfusate available no significant action on the blood pressure of the rat could be observed. The perfusate collected under these conditions, as well as the fluid collected immediately after inserting the needle (Table 4) contained a substance with properties indistinguishable from those of bradykinin with the methods used. Much more bradykinin (approximately 500 milliunits) is required to induce an observable depressor response in the rat blood pressure than is required to induce an observable response of the rat uterus or duodenum (approximately 5-10 milliunits).

*III. **Bioassay of Perfusate Collected During Stimulation of the Distal Portion of Transected Dorsal Roots.***—*In 3* subjects undergoing rhizotomy performed for intractable pain, the subcutaneous tissues underlying the skin subserved by the dorsal roots being sectioned were perfused and

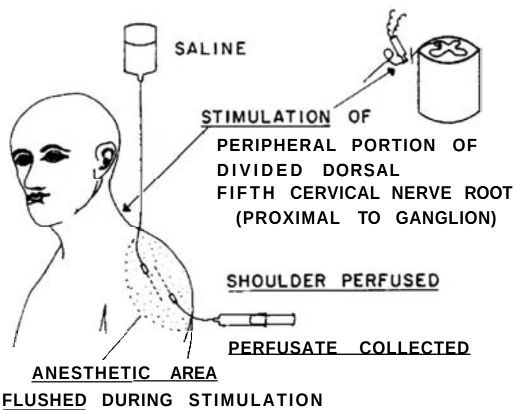


Fig. 6.—Method of collecting subcutaneous perfusate before and during antidromic vasodilatation in the skin induced by stimulation of the distal portion of a transected dorsal root after rhizotomy performed for intractable pain.

control specimens of perfusate collected before cutting the nerves. When the roots had been transected, their distal portion was stimulated electrically, and samples of perfusate again collected (Fig. 6). In 2 experiments, the epaulet area of the shoulder was perfused while roots C_4 and C_5 were stimulated. In the third subject the skin of the forearm was perfused while roots C_8 and T_1 were stimulated. Bioassay of the perfusate indicated that perfusate collected during such stimulation contained a substance that is similar to the substance found in subcutaneous perfusate during flare resulting from noxious stimulation of the skin. Other substances which remain at present undefined also are present in appreciable amounts (Fig. 7).

*IV. **Similarity of the Properties of the Substance Formed During Flare Induced***

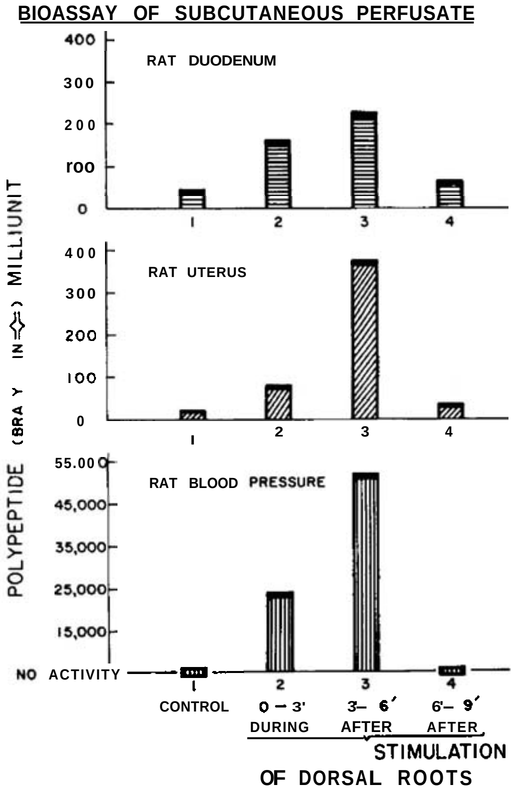


Fig. 7.—Bioassay of subcutaneous perfusate collected before and during stimulation of the distal portion of a transected dorsal root in man, demonstrating, that a pharmacodynamically active material is elaborated in the periphery.

by Noxious Stimulation to Those of a Substance *Formed* by Incubation of Cerebrospinal Fluid *with* Globulin.—Comparison of the properties of the substance formed during flare induced by noxious stimulation with those of the substance formed by incubation of cerebrospinal fluid with plasma globulin by means of quantitative assay with the rat uterus, duodenum, and blood pressure indicated that the 2 substances closely resemble each other or are identical (Tables 1 and 5).

V. The Neuroanatomic Basis of Cutaneous Vasodilatation (Flare) Evoked by Noxious Stimulation of *the* Skin.—A. Transection of Peripheral Nerve: Two subjects with accidental transection of a peripheral nerve became available for study. A bullet wound damaging the brachial plexus sustained 2 months before study in

Table 5.—Units of "Unknown" Substance Required to Induce Effect Equal to One Unit of Bradykinin

	Rat Duodenum	Rat Uterus	Blood Pressure of Rat
CSF + globulin	1	19	0.03
Reflex flare perfusate	1	15	0.03
Thermal injury perfusate	1	1	No action
Initial perfusate	1	0.8	No action

This table is based on the data provided in Tables 1, 2, and 4. One unit of the "unknown" substance was defined as the amount of specimen necessary to induce a relaxation of the rat duodenum (in a solution containing 0.02 gm. $CaCl_2$/L) equal to that induced by 1 unit of bradykinin. The ratios for the CSF + globulin mixture and for the subcutaneous perfusate collected during the onset of "reflex flare" induced by noxious stimulation are similar, suggesting that the activity of the specimens results from the same substance. Since these ratios differ greatly from unity, it is evident that the active agent is not bradykinin. The specimens of subcutaneous perfusate collected at the beginning of the perfusion ("initial perfusate") and the specimens collected directly underlying a region of thermal injury contained another substance that was relatively more active on the uterus, and less active on the blood pressure. Since the observed ratios approximate unity, the active substance in these perfusates may be bradykinin.

one subject and a bullet wound involving the common peroneal nerve sustained 12 days before study in the second had resulted in areas of cutaneous anesthesia. It was demonstrated that flare did not occur in response to noxious stimulation (intradermal injection of 0.05 cc. histamine, 1:1,000) within these anesthetic areas. Also, analysis of subcutaneous perfusate revealed no increase over control levels of pharmacodynamically active substance in the regions adjacent to sites of noxious stimulation.

B. Sympathectomy: The possibility that the sympathetic nervous system participates in flare resulting from noxious stimulation was examined by study of a subject whose forearm was deprived of sympathetic innervation. Twelve years before the subject had been treated for Raynaud's syndrome by bilateral upper thoracic sympathectomy and bilateral anterior rhizotomy of the first thoracic nerve. The sympathetic chain was divided below the third ganglion, and the second and third intercostal nerves were resected. The stellate, second, and third ganglia and the central attachment of the first thoracic root were left intact. The first thoracic anterior root was transected bi-

laterally, leaving the posterior roots intact. The absence of sympathetic innervation in the region of the forearm studied was demonstrated by the failure of noxious stimulation to evoke sweating, as shown by the starch-iodine method and by the failure of noxious stimulation to cause changes in the electrical resistance of the skin. Nevertheless, flare indistinguishable from that observed on the patient's other, intact, forearm resulted from noxious stimulation. Analysis of subcutaneous perfusate demonstrated the usual increased amounts of pharmacodynamically active substance during the development of the flare.

C. Section of Dorsal Roots Proximal to Ganglia: After noxious stimulation of the skin by intradermal histamine, a typical flare and the usual increase of the pharmacodynamically active substance was found in subcutaneous perfusate, in a subject in whom, one year previously, the dorsal roots that had served the region studied, had been sectioned between the spinal cord and the dorsal root ganglia (rhizotomy of T_1 through T_7). Transection proximal to the ganglia, while depriving of sensation the region subserved by the nerves, does not lead to the degeneration of the peripheral afferent fibers.[116]

In another subject studied before and after rhizotomy (C_4, C_5) proximal to the ganglia, it was observed that the intensity of the flare following intradermal injection of histamine was reduced in the anesthetic region during the first week after sectioning, but thereafter gradually increased, returning approximately to its usual intensity in approximately 20 days. Assay of subcutaneous perfusate collected during this period indicated that the amount of the defined substance observed in the perfusate closely paralleled the magnitude of the flare. During the first 4 days after sectioning the roots, when the erythema was only faintly pink in color, no observable increase of activity in the perfusate occurred following noxious stimulation. After the fourth day, the amount of substance assayed after noxious stimulation increased each day as the erythema became more pronounced. The initial depression of the magnitude of flare in this subject probably stemmed in part from electrical stimulation of the roots during the surgical procedure and may not occur in the absence of such stimulation.

D. Extirpation of Dorsal Root Ganglia: Whether flare would occur in the subserved areas after removal of the ganglia of the dorsal roots (C_8, T_1, T_3) was systematically investigated in one patient. Two days after removal of the ganglia the intradermal injection of histamine into the anesthetic region evoked a flare response in the anesthetic region. It was already smaller in area than that evoked in the patient's intact arm and the color of the "red reaction" was less intense. A moderate amount of pharrnacodynamic activity was observed in the perfusate collected during the development of flare. Over the next 10 days, however, the intensity and the area of the flare in the anesthetic region progressively diminished until only a bleb with no surrounding flare formed at the site of histamine injection (Figs. 8, 9, and 10). At this time bioassay of perfusate from the regions adjacent to the site of histamine injection in

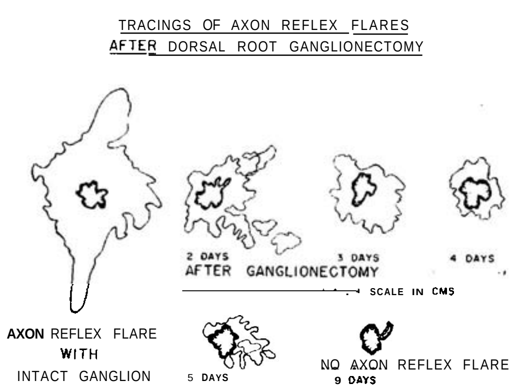


Fig. 8.—Tracings of wheal and flare induced by intradermal injection of 0.05 cc. histamine phosphate (1:1,000). The control tracing at the left was obtained before, and the remainder of the tracings after, extirpation of dorsal root ganglia performed for relief of intractable pain. The postganglionectomy flares were induced within the cutaneous zone of anesthesia resulting from ganglionectomy. Note that flare can still be evoked for several days after ganglionectomy, but the response gradually dwindles and after 9 days is absent.

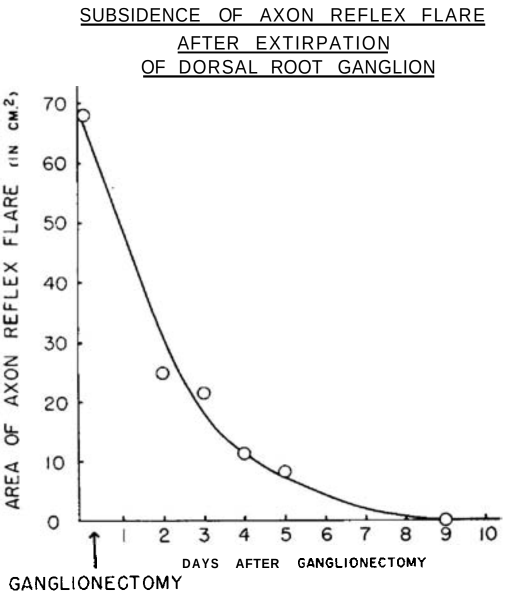


Fig. 9.—Demonstration of the effect of ganglionectomy. The areas of flare shown in Figure 11 are plotted as a function of the time after dorsal root ganglionectomy. Although the flare gradually dwindled and disappeared after ganglionectomy the area of the wheal did not change from that evoked when the region was normally innervated.

the anesthetic region showed no release of the substance that depresses rat blood pressure, contracts rat uterus, and relaxes rat duodenum. Flare evoked by noxious stimulation in adjacent skin with normal sensation stopped at the borders of the anesthetic area (Fig. 11, see page 637).

Whereas the area of the erythema progressively became smaller during the period when the dorsal root fibers were degenerating, the area of the wheal remained unchanged even when the afferent nerve degeneration apparently was complete (see Fig. 8). However, even though the area of the wheal was unchanged, the color of the wheal was pink rather than white (see Fig. 10).

Comment

The Neuroanatomic Basis of Cutaneous Vasodilatation (Flare) Evoked by Noxious Stimulation of Human Skin.—*As* cited above, the early studies of Bruce,[91] Ebbeke,[90] and Krogh,[29] implicated the nervous system and particularly the afferent nerves in vasodilatation resulting from noxious stimulation by showing that it is greatly reduced by local anesthetics and by transection of the nerves serving the region. Bruce[91] specifically demonstrated the importance of afferent fibers by showing that the vasodilator response to noxious stimulation in the rabbit conjunctiva was attenuated after trigeminal ganglionectomy. Müller[1] noted that the flare response was absent in anesthetic regions of the skin after peripheral nerve injuries. Lewis and Grant[2,6] established that cutaneous flare can be evoked by noxious stimulation solely on the basis of "axon reflexes" by showing that the capacity to exhibit "flare" after noxious stimulation of human skin persisted for 6 or 7 days after transection of the relevant peripheral nerve and then was lost. These observations did not directly demonstrate that it is the afferent fibers that are implicated, since the regions studied lacked autonomic and efferent innervation as well.

The studies reported here of patients with peripheral nerve injuries confirmed the observations of Lewis and Grant with similar subjects. The observations of the patients deprived of sympathetic innervation in the region studies demonstrated that the sympathetic innervation is not essential to the flare reaction to noxious stimulation, again in confirmation of Lewis and Grant.

Cutaneous reactions to injury in anesthetic regions of human subjects with transection of dorsal root fibers between the ganglion and the cord or with dorsal root ganglionectomy apparently have not been studied previously. The observation of patients with transection between the ganglion and the cord established that interruption of the link with the cord without degeneration of the peripheral fibers does not abolish flare in the anesthetic region of the skin. In contrast, it was established that extirpation of the dorsal rwt ganglia *does* abolish the flare response to noxious stimulation in the segment subserved after 7-9 days, during which the periphery of the flare progressively shrinks and the intensity of the

erythema gradually fades. Since dorsal root ganglionectomy is apparently followed by degeneration of afferent fibers only, the persistence of the flare reaction after transection of the dorsal roots between the ganglion and the cord and its gradual disappearance after extirpation of the ganglia present direct evidence that it is the integrity of certain of the afferent fibers stemming from the dorsal root ganglion cells that is essential to the phenomenon of diffuse cutaneous vasodilatation (flare) evoked by noxious stimulation of human skin. Also, since the efferent and autonomic innervation in the subject studied was intact at a time when the flare response was totally absent, it also can be concluded that not only are these fibers not essential to the phenomenon, but they do not contribute significantly to it.

Celander and Folkow[98,99] inferred that of the dorsal root afferent fibers, it is only those subserving pain that participate in the flare response. They showed that there is close agreement between the initiation of activity in afferent fibers which respond only to stimuli that are "damaging" and the initiation of the flare response. Benjamin[117] also observed close agreement between the threshold for the flare response and the threshold for pain resulting from thermal stimulation in human subjects.

The brief persistence of the capacity to evoke a flare response after ganglionectomy and the gradual fading of this capacity during the period when the peripheral branches of the afferent axons are degenerating distally support the inference of Lewis and Grant that the development of flare in response to noxious stimulation is possible on the basis of reflexes taking place within the branches of single afferent axons. However, in light of recent evidence relating to the "dorsal root reflex" it is necessary to consider the possibility that in intact subjects the flare response stems not only from axon reflexes but also from reflexes in which impulses pass orthodromically to the central nervous system and there give rise to impulses which pass antidromically to the periphery.

In 1891, Gotch and Horsely[118] observed by means of a galvanometer that stimulation of peripheral nerve-evoked reflex discharges in the central portion of dorsal roots after transection between the ganglion and the cord. They concluded that "when a spinal center is thrown into activity, a portion of its energy flows as a discharge backwards down the posterior roots as well as forwards down the efferent fibers of the anterior roots and upwards and outwards along internuncial fibers to the next center." These "dorsal root reflexes" were again studied in the cat in the 1930's by Barron and Matthews,[119] who concluded that impulses originating from sensory receptors enter the cord and give rise to antidromic discharges which pass out the dorsal roots to the periphery. A few of these impulses "appeared to originate in intraspinal neurons." They considered the possibility that some of the fibers conducting the centrifugal impulses terminated in endings associated with blood vessels: "It seems possible that many of the fibers carrying antidromic sensory impulses may go to blood vessels and be responsible for Bayliss' antidromic vasodilatation."

Although no evidence could be presented to support it, they also suggested that the "dorsal root reflex" might be influenced by activity within the central nervous system: "Unlike the classical example of the axon reflex (Bayliss, 1901) the branching here takes place within the cord. The peculiar properties of the central conduction in this path might enable the central nervous system to influence the reflex." After careful study of homolateral and contralateral reflex discharges occurring in the dorsal roots after stimulation of the saphenous nerve, Toennies[120,121] concluded that "fibers from the dorsal root ganglia make central synaptic connection with internuncial neurons of the cord in such a way that they can be reflexly excited with a resulting discharge of impulses toward the periphery of the body."

More recently it has been shown by Wall[122] that single shocks applied to dorsal

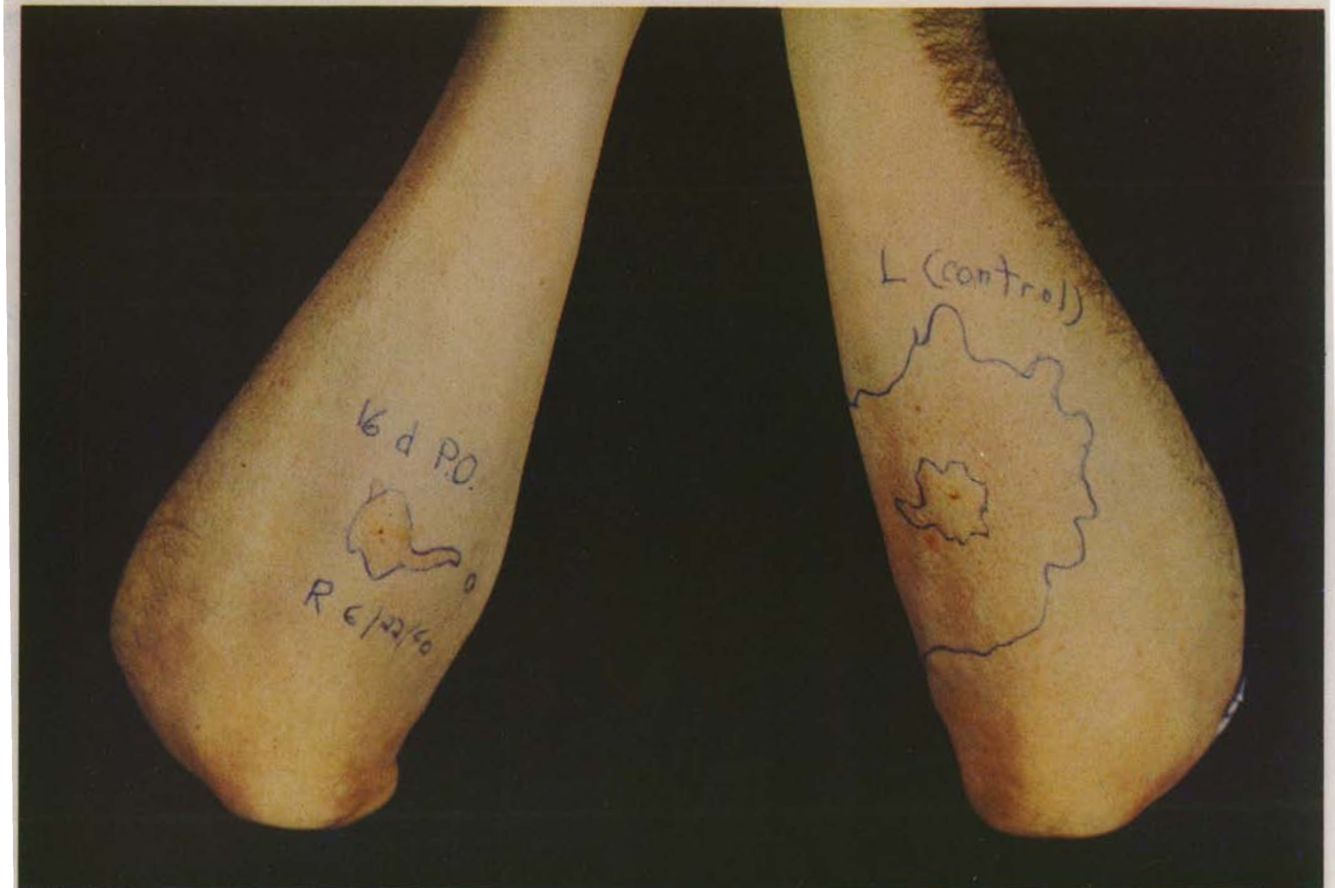


Fig. 10.— Demonstration of the effect of ganglionectomy. Colored photograph of responses to intradermal injection of 0.05 *cc.* histamine phosphate (1:1,000). Histamine was injected simultaneously in the skin of the normally innervated arm (L) and in the cutaneous zone of anesthesia (R) resulting from extirpation of a dorsal root ganglion. The photograph was made 16 days after ganglionectomy.

roots resulted in a major increase in the excitability of the terminal arborizations of the afferent fibers within the dorsal horn of the cord. In contrast, excitability within the arborizations of proprioceptive fibers in the ventral horn was only slightly enhanced after orthodromic stimulation.

Investigations of the link between orthodromic and antidromic activity within the dorsal root fibers[118-125] have focused exclusively on electrical events, but conceivably a neurohumoral substance (perhaps identical with the vasodilator mediator released in the periphery during dorsal root stimulation) released from the endings of the branches within the terminal arborization could increase excitability, thereby promoting and perhaps initiating antidromic activity.

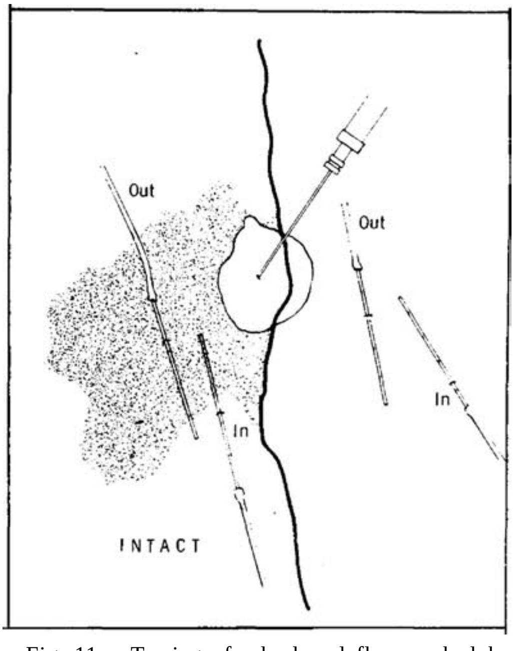


Fig. 11.—Tracing of wheal and flare evoked by intradermal injection of histamine phosphate (0.05 cc.) at the perimeter of a cutaneous zone of anesthesia resulting from extirpation of 3 dorsal root ganglia 18 days previously. The bleb (shown as a white area in the drawing) extended partly into the region of normal sensation and partly into the anesthetic region. The flare reaction (shaded area) extended widely in the normally innervated region but stopped at the border of the anesthetic region. Perfusate from the intact region increased in its pharmacodynamic activity during the onset of flare as described in the text. In contrast, perfusate from the analgesic zone did not increase above a low, control level of activity.

The Neurohumoral Basis of Cutaneous Vasodilatation (Flare) Evoked by Noxious Stimulation of Human Skin.—That a mediator substance is implicated in this neurogenic vasodilatation is suggested by the readily observable latent interval (15-30 seconds) between noxious stimulation and the onset of the flare response, and the even longer period (5-10 minutes) that elapses before the response is maximal. Lewis and Marvin[3] showed that both the latency and the duration of the flare response can be increased by occluding the circulation of the blood to the region being studied. The observations reported herein (see Fig. 1) demonstrated that intradermal injection of subcutaneous perfusate collected during the onset of a flare reaction evoked by noxious stimulation of the skin results in pain sensation and evokes a flare reaction surrounding the site of reinjection. These observations support the conclusion that this neurogenic vasodilatation is mediated through the elaboration of a chemical substance. They also indicate some of the properties of the mediator substance: (a) it induces burning pain; (b) it evokes a flare response, and (c) it is relatively stable—since the perfusate retained its activity during the period between its elaboration and its reinjection into the skin.

Bioassay of subcutaneous perfusate demonstrated that during the period of active vasodilatation of the flare reaction a substance with potent hypotensive properties is elaborated. The fact that no such material occurred during reactive hyperemia demonstrated that it is not a secondary consequence of vasodilatation per se and indicates that the substance does not escape into the tissue from leakage through dilated and permeable vessel walls. Rather, it suggests that the substance itself acts to induce the vasodilatation. A predictable ratio of activity on several assay systems makes it seem

likely that the active perfusate gains its properties from a single substance.‡

The defined properties of this hypotensive substance indicate that it is a vasodilator polypeptide. The evidence that its chemical structure is that of a polypeptide rests on the observation that brief incubation with chymotrypsin destroys its activity. Better definition of the chemical nature of the substance must await preparation of highly purified samples and appropriate characterization by biochemical methods.

The subcutaneous perfusate collected from a region of antidromic vasodilatation induced by stimulation of transected dorsal roots contained large amounts of a pharmacodynamically active substance with properties qualitatively similar to those of the substance present in perfusate collected during flare induced by noxious stimulation of the skin. The greater activity of this perfusate on the uterus and the blood pressure relative to its activity on the duodenum, however, indicate that the perfusate collected during electrical stimulation of the dorsal roots contains appreciable amounts of other substances as well.

"Neurokinin."—Since the pharmacodynamic properties of the substance observed in subcutaneous perfusate during the onset of flare evoked by noxious stimulation apparently are not identical with those of other substances heretofore known to occur in the body, it seems appropriate to suggest a name for it. "Neurokinin" is suggested. "Neuro-" because of its association with neurogenic vasodilatation and because an enzyme capable of forming it when incubated with plasma globulin has been demonstrated to occur in cerebrospinal fluid, and, "-kinin" because of its similarity to bradykinin and to plasma kinin. As mentioned above, the defined properties of neurokinin also closely approximate those of oxytocin, a potent polypeptide known to occur within the central nervous system. Qualitatively, they have much in common. When equated in terms of the response of the isolated rat duodenum, however, the ratios of their pharmacodynamic activities on several bioassay systems indicate that neurokinin differs from oxytocin in its greater hypotensive action and its lesser uterus-contracting potency.

These studies do not define the origin of neurokinin observed in subcutaneous perfusate during flare. Conceivably the polypeptide is formed within the neuron and is extruded during nerve activity. However, the demonstration that perfusate collected immediately after noxious stimulation is increased in potency following 3-minute incubation with globulin, indicates that the proteolytic enzyme capable of forming neurokinin is present in the extracellular fluid at that time. Thus, it is either a proteolytic enzyme activator or the enzyme itself that is released. The capacity of cerebrospinal fluid to form neurokinin when incubated with plasma globulin indicates that cerebrospinal fluid contains an enzyme capable of liberating the polypeptide. This enzyme may act through splitting peptide linkages or it may catalyze the release of the polypeptide from some weaker linkage. It is tempting to speculate that the neurokinin-forming enzyme is an intraneuronal protease that is activated during heightened cellular metabolism. Proteolytic enzymes capable of acting at a neutral pH occur in the brain,[126] and reversible chemical changes in rapidly frozen brain homogenate have been described which suggest that stimulation of the brain induces a state of excitation in which the rate of protein breakdown increases, proteolytic enzymes are activated, and partial protein denaturation occurs.[127-129] However, the hypothesis of the release of a neuronal protease during flare would require an enzyme to pass out of the cell boundaries during excitation. Whether or not proteins can pass out of

‡ Since it is highly probable that the perfusate collected under these circumstances contains at least small amounts of a variety of physiologically active materials, the predictable ratios of activity observed also suggest that the potency of the substance on the assay systems used is so great that its action outweighs the influence of other substances present.

nonpathological nerve cells during excitation is still uncertain.

The Possible Significance of the Mediator "Neurokinin."—Bradykinin, a polypeptide apparently closely related to neurokinin recently has been prepared in a highly purified form and a chemical structure has been proposed for it.[130] A polypeptide with many of its properties has been synthesized.[131] On a molar basis, bradykinin is a more potent vasodilator than acetylcholine.[132] When equated as equiactive on the rat duodenum, neurokinin is 35-50 times more potent than bradykinin in its hypotensive action (rat blood pressure). By acting as a mediator for the flare reaction evoked by noxious stimulation neurokinin may act as an agent for promoting inflammation during exposure to noxious stimulation, contributing to the protection of the organism as a whole by sacrificing the integrity of a part.

In addition to its role in neurogenic vasodilatation evoked by noxious stimulation, it is conceivable that neurokinin may also participate in local vasomotor control within some portions of the central nervous system, since neurokinin-forming enzyme has been found to be increased in cerebrospinal fluid after convulsions in man[111] and in perfusate from the cerebral ventricles during stimulation of the brain or of afferent nerves of laboratory animals,[112] conditions under which increased cerebral blood flow is known to occur.[133,134]

Recent studies using more sensitive methods than were used previously[135] have indicated that all specimens of human cerebrospinal fluid, whether from subjects with disease of the central nervous system or from those free of disease, contain at least small amounts of the enzyme capable of forming this polypeptide. However, in subjects free of disease the amount present is so small that its presence can be determined only when the sensitivity of the bioassay method is maximal. The fact that it is the enzyme and not the polypeptide that can be observed in cerebrospinal fluid of patients with disease of the central nervous system and perfusate of the cerebral ventricles during electrical stimulation may be a consequence of the small amount of protein present in cerebrospinal fluid. Conceivably, there is sufficient globulin precursor present in the extracellular fluid of the brain to form amounts of the polypeptide that are physiologically active but are too small to be detected by bioassay. In pathological states and after massive stimulation this endogenous substrate may be exhausted. In these conditions enough of the enzyme apparently is released to form detectable amounts of polypeptide when globulin is added.

The possibility exists that neurokinin and neurokinin-forming enzyme may be released within the central nervous system and influence neural activity there. Dale pointed out in 1935[136] that since the bipolar afferent neurons implicated in cutaneous flare do not synapse until they are within the central nervous system, the vasodilator substance released in the periphery during antidromic vasodilatation may also be a transmitter at the central synapse of afferent neurons. This reasoning is dependent upon the assumption that the fibers which conduct afferent impulses are the same as those responsible for antidromic vasodilatation. Feldberg[137] concluded that "there is a high probability that this is so, and therefore any information about the nature of the transmitter of antidromic vasodilatation touches at the root of the problem of central synaptic transmission." Hellauer and Umrath[73] specifically suggested that the mediator substance of antidromic vasodilatation induced by stimulation of dorsal roots is identical with the central synaptic transmitter substance of afferent nerves. Experimental evidence for the central release of a mediator substance was presented by Häusler and Sterz,[138] who reported that stimulation of afferent nerves releases a histamine-like substance into perfusate of the spinal cord of the frog.

However, there are difficulties with the postulate that the mediator substance of antidromic vasodilatation serves as a synaptic transmitter for the neurons of the central

nervous system. The long latency and long duration of antidromic vasodilatation indicate that the mediator is a substance that is formed and destroyed too slowly to act as a synaptic transmitter.[139] On the other hand, Habgood's[107] experiments indicate that a substance released by stimulation of cutaneous nerves in frog skin can lower the threshold for neuron firing and may initiate spontaneous impulses. Also, Wall's[122] observations indicate that orthodromic stimulation of certain afferent fibers can lead to heightened excitability in the general region of the cord in which these fibers synapse.

Thus, the slow formation of neurokinin and its stability suggest that if it is released within the central nervous system, it acts not as a "synaptic transmitter" which would require extremely rapid release and destruction, but as a slow-acting substance that heightens excitability within the central nervous system over long periods.

Inflammation.—A number of events associated with inflammation can be interpreted as indicating that antidromic impulses in afferent nerves result in the peripheral release of humoral agents that damage tissue or heighten tissue vulnerability, and influence invasion, survival, and growth rate of micro-organisms.

In patients with tabes dorsalis, ecchymosis of the skin of the involved areas sometimes occurs after prolonged periods of pain associated with inflammation or injury of the dorsal roots.[140]

It has been demonstrated in laboratory animals that lesions in the intestines can be produced by stimulation of the peripheral portion of the transected splanchnic nerve[141] or of the dorsal roots through which pass the afferent fibers of the splanchnic nerve.[142]

Sectioning of that portionof thetrigeminal nerve which includes the fibers subserving sensation of the lips, is fallowed by the occurrence of herpes vesicles in the lips in a high percentage of instances, especially when the section is made close to the ganglion.[143] These lesions do not occur if the lip area has been denervated previously.[144] Also, if the afferent fibers are cut within the brain stem itself, or at some distance from the ganglion, the incidence of herpes lesions is greatly reduced.[145] These observations suggest that surgical trauma during section of the nerve fibers initiates antidromic impulses that create an environment in the skin favorable for the activation of latent herpes virus.

Recently, it was observed that subcutaneous perfusate of tender and aching regions of the head during vascular headache of the migraine type contained a substance with properties indistinguishable from those defined in the present studies for neurokinin. The concentration of the substance observed in the perfusate was related closely to the severity of the headache attack.[146]

Other studies from this laboratory have indicated that changes mediated by peripheral neural pathways and possibly involving the release of neurohumoral agents can enhance the inflammation reaction, although it has not yet been possible to define whether the relevant neurons are dorsal root or autonomic fibers.[147]

Neurohumoral Mediators and Pain.—The findings of the present study and of earlier studies from this laboratory[7] reveal the need for reassessment of the role in painful states of neurohumoral substances acting in the local environment of the nerves, both in the periphery and in the central nervous system.

The pain threshold is reached when a stimulus just becomes injurious to tissue.[148,149] When the stimulus is radiant energy, the threshold closely approximates the temperature at which partial denaturation of protein begins.[150]

Although under the usual conditions of health the pain threshold is relatively uniform and stable, it can change when the local internal environment is altered. Under the protective influence of extensive edema, the pain threshold may rise. Conversely, it may fall when the local environment is altered by pathological processes which promote protein denaturation, heightened catabolism, or increase in the excitability

of the nerve endings subserving the sensation of pain.

While it is probable that pain receptors can be activated directly by physical or chemical features of noxious stimulation, it is also possible that a locally released endogenous substance acts to initiate impulses in fibers subserving pain. Histamine has been considered to be such a mediator substance.[151-154] Parrot [101] recently concluded that "it may be possible to assume the existence of histaminoceptive fibers; other substances, however, especially acetylcholine, may participate in the stimulation of these fibers." Histamine is released from the tissue mast cells under conditions of even mild noxious stimulation,[155] and histamine results in burning pain and itching when injected in great dilution.[151] It is not possible to conclude, however, that histamine is the sole or principal endogenous agent that evokes activity in pain receptors when tissue is damaged.

The complex biochemical changes that take place in regions of injured skin result in at least a transient formation of a number of substances capable of lowering the pain threshold by promoting protein denaturation and otherwise heightening catabolism, thus reducing the amount of external stimulation required to "injure" tissue and induce pain. Some of these substances also may be capable of altering the excitability of the nerve endings subserving pain sensation and of initiating impulses in them.

A number of potent pharmacodynamic substances have been demonstrated to be present in inflammatory exudates.[156-163] It has long been known that proteolytic enzymes are activated under conditions of tissue injury.[164] They are also activated during allergic reactions [165] and probably are implicated in the mechanism of histatnine release.[166] Adenosine and adenylic acid are released from burned tissues.[167] Increased hydrogen ion concentrations occur in damaged regions, and small blood vessels can be dilated by focal accumulation of acid solutions.[168] Keele and his associates have defined a pain-producing substance that is formed when blister fluid is brought into contact with glass.[169,170] It has many of the properties of bradykinin. Menkin has identified several potent substances (leucotoxin, necrosin, exudin) in inflammatory exudates.[171] Benjamin [172] has postulated that noxious stimulation releases intracellular potassium, and it is known that injection of isotonic solution of potassium chloride in great dilution is painful.[168] Further, it is well known that injection of hypertonic or hypotonic solutions is painful and that tissue injury results in deviation from isotonicity and alteration in osmotic pressure.[168] Recently, Rocha e Silva [173] observed that bradykinin, which induces pain when introduced into the skin,[174] was elaborated in subcutaneous pouch fluid under milder conditions of noxious stimulation than were required to release histamine.

Thus, several physical and chemical changes occur in regions of tissue injury that could influence pain threshold and perhaps activate pain endings. Some of these changes result in the elaboration of substances that are painful and result in flare reactions when introduced into the skin in small amounts. However, it is not yet possible to decide whether or not any one substance in the region of noxious stimulation predominates in initiating nervous activity. In short, several "pain substances" may participate in initiating activity in nerve endings.

Since the studies herein reported demonstrate that perfusate collected from a region of reflex vasodilatation induced by noxious stimulation has the capacity to induce pain and evoke a flare response when reinjected intradermally, it can be concluded that the neurogenic mediator substance for this vasodilatation has the capacity to initiate activity in pain fibers and thus may be considered a "pain substance."

Referred Pain.—Stimulated by the familiar bedside phenomenon that hyperalgesia of the skin may occur in zones of referred

pain without obvious vasodilatation, Hardy, Wolff, and Goodell[175] attempted to ascertain the nature of hyperalgesia in general and that associated with referred pain in particular. They injured the skin by burning, by faradic stimulation, and by the intradermal administration of chemical agents, including histamine. After such injuries, hyperalgesia to pin prick in the large areas including the resulting flare zones was observed. It was noted that the hyperalgesia extended as well into nonerythematous regions some distance proximally as well as distally from the area of flare. The lowered pain threshold of the site of injury to the skin was noted and the alteration was termed primary hyperalgesia. But at this time no lowering of the pain threshold in the area of vasodilatation as defined by the radiant energy method in the flare zone evoked by noxious stimulation was observed. Subsequently[7] it has been found that an error had been made and that a transient lowering of the pain threshold does occur in the flare zone during the phase of active vasodilatation. The maximal lowering of pain threshold in a zone of flare persists only for a few minutes during the onset of flare; thereafter, sometimes gradually and often rapidly, the pain threshold returns to normal even though the skin remains reddened.

Since it is now clear that the pain threshold is lowered for a time during the onset of flare evoked by noxious stimulation, it is evident that there is both a primary and a secondary hyperalgesia in the areas surrounding the site of injury to the skin. Unless there is extensive local edema, there is a striking lowering of pain threshold at the site of the injury. This lowering of the pain threshold represents primary hyperalgesia. There is a less striking and transient lowering of pain threshold in the area of the flare which also must be considered primary hyperalgesia. In addition there is a zone of hyperalgesia to pin prick which extends beyond the site of injury and beyond the flare zone in which there is no vasodilatation and no lowering of the pain threshold. This represents a zone of secondary hyperalgesia, a term used to describe the state in which all stimulation at threshold or above, whether by pin, by heat, or other noxious stimulation is experienced as more intense than elsewhere even though pain threshold is not lowered.

The primary hyperalgesia in the area of flare induced by noxious stimulation is probably due to the peripheral liberation in the tissue of a pain threshold lowering substance. The secondary hyperalgesia in the zone extending beyond the flare is apparently due to heightened excitability within the neuraxis. It may also involve neurohumoral mediators, acting within the central nervous system.

Lewis and Kellgren,[176] studying the hyperalgesia that occurs in cutaneous regions of "referred pain" after noxious stimulation of viscera or muscle, were much impressed by the observation that although pain seeming to emanate from the skin occurred immediately, the onset of hyperalgesia in the cutaneous area of referred pain was delayed. The onset of hyperalgesia sometimes did not occur until after the painful sensations had stopped. They concluded that this hyperalgesia arises "not through the central nervous system, but through a purely peripheral mechanism." Weddell, Sinclair, Feindel, and Falconer,[177] and Hardy, Wolff, and Goodell,[175] who studied the intervertebral ligaments as a source of pain, also observed that the interval between noxious stimulation of deep structures and maximal cutaneous hyperalgesia sometimes was as long as 15 minutes and long outlasted the pain.

There is evidence that a mediator substance is sometimes released peripherally in regions of referred pain. The grossly apparent hyperemia in the conjunctiva that follows noxious stimulation within the nose,[178] and that which sometimes occurs in regions of referred pain in the skin[148] suggest that a substance, perhaps neurohumoral, is released locally in the regions of cutaneous hyperalgesia. However, not all of the features of "referred pain" can be accounted

for by the peripheral release of a pain threshold lowering substance. For example, under certain conditions pain may be "referred" to a completely anesthetized area.[179-181] In the case of widespread referred pain in the head following prolonged noxious stimulation of a tooth, the pain may be abruptly ended by procainization of the damaged tooth, but not by procainization in the area of referred pain.[182] Furthermore, in most instances of referred pain studied in this laboratory there has been neither reddening nor lowering of pain threshold (by thermal pain threshold measurement) in the area of referred pain, even though the pin prick is felt as sharper.[149,183]

Sinclair, Weddell, and Feindel[184] postulated that referred pain is dependent upon "branching" of single axons, one branch passing to the site of noxious stimulation and another to the site to which the pain is referred. They concluded that both the central interaction of neural impulses and the actions of an agent released at the termination of the nerves are implicated in referred pain, "the first being initially more important than the second, but the second increasing in importance with time, and in the latter stages predominating."[184] The formulation of these authors postulates that in all instances of referred pain both peripheral and central elements are present. However, as cited above, in most instances of referred pain studied in this laboratory, the peripheral element has not dominated and has not always been demonstrable at the time these observations were made.

It is therefore recommended that when vasodilatation is present in the hyperalgesic zone of an area of referred pain, the hyperalgesia be termed "primary" during the active phase of vasodilatation. When no vasodilatation is present and the pain threshold is not lowered, it should be termed "secondary hyperalgesia."

Summary and Conclusions

The subcutaneous tissues of an area on the volar surface of the forearm were perfused with normal saline. Vasodilatation in the area was then induced by pinching and crushing the adjacent skin. Perfusates collected (a) during control periods when no flare reaction was present and (*b*) during the onset of cutaneous flare were reinjected intradermally on the back. Perfusate collected during the onset of flare predictably induced burning pain and larger, more intense, flare reactions than did control specimens.

Perfusate collected during the onset of cutaneous flare induced by noxious stimulation of the skin adjacent to the perfusion site acquired or greatly increased its capacity to contract isolated rat uterus, relax isolated rat duodenum, and depress the arterial blood pressure of the rat. The pharmacodynamic properties of the perfusate could be stabilized by boiling and destroyed by incubation with chymotrypsin. These properties stemmed from a vasodilator polypeptide in many ways resembling bradykinin, kallidin, and oxytocin, but were not identical with the properties of these or several other related substances. Because of its association with neurogenic vasodilatation, "neurokinin" was suggested as a descriptive label.

Subcutaneous perfusates collected before and during vasodilatation after occlusion of the circulation of the arm were then compared. Perfusate collected during reactive hyperemia showed no increase in its pharmacodynamic activity above control values, indicating that the above described properties of the perfusate did not occur as a consequence of vasodilatation per se.

The distal portions of transected dorsal roots in man were stimulated while the subcutaneous tissue underlying the region of skin previously subserved by the roots was perfused. The perfusate collected during stimulation greatly increased in its pharmacodynamic properties. It was augmented in its capacity to relax the rat duodenum, contract the rat uterus, and depress the rat blood pressure, suggesting that it contained neurokinin. However, the perfusate also

contained, as well, significant amounts of other, undefined substances.

It was inferred that the defined substance or complex termed neurokinin, is a mediator of neurogenic vasodilatation in the skin evoked by noxious stimulation. Its powerful hypotensive action, and the good agreement between the amount of pharmacodynamic activity in the perfusate and the degree of flare observed, suggest that neurokinin may be the principal mediator of this vasodilator response.

About a week after functional discontinuity of peripheral nerve, no flare and no release of pharmacodynamically active agents could be demonstrated in subcutaneous perfusate of anesthetic regions following noxious stimulation of the skin.

Elimination of sympathetic nerves did not prevent the development of flare after noxious stimulation of the skin and did not interfere with the occurrence of a pharmacodynamically active agent in subcutaneous perfusate collected during the phase of active vasodilatation.

One year after section of the dorsal root fibers between the dorsal root ganglia and the spinal cord (leaving intact the ganglia and the peripheral fibers) the usual flare reaction and the presence of pharmacodynamically active agents in the perfusate following noxious stimulation of the skin in the anesthetic region could be demonstrated.

Cutaneous flare evoked by noxious stimulation of the skin is not dependent upon efferent fibers emerging from the spinal cord, since transection of the dorsal root between the ganglion and the cord would be expected to result in peripheral degeneration of such hypothetical efferent fibers (and absence of the flare reaction) within a period of a few weeks.

During the first week after excision of dorsal root ganglia the periphery of the flare resulting from standard noxious stimulation of the skin became progressively smaller and the erythema less intense until after 7-9 days the flare was absent and only a highly circumscribed reaction at the site of injury could be evoked.

Cutaneous flare evoked by noxious stimulation of human skin is thus the result of reflex activity of neurons with cell bodies located in the dorsal root ganglia and can occur solely on the basis of axon reflexes in the peripheral branches of single afferent nerves.

The neurohumoral mediator of the flare reaction is a "pain substance" that induces lowering of the pain threshold transiently in the zone of vasodilatation surrounding sites of noxious stimulation. It also causes the lowering sf the pain threshold in the skin associated with referred pain when a phase of cutaneous vasodilatation and primary hyperalgesia occurs. This primary hyperalgesia thus supplements the secondary hyperalgesia.

The elaboration in the periphery of the neurohumoral mediator associated with antidromic reflex activity in afferent nerves is a contribution of the nervous system to the inflammatory reaction.

We express our appreciation to Dr. Bronson Ray for his help with these studies. We are especially grateful to him and to Dr. Russel H. Patterson, Jr., for the privilege of studying patients with intractable pain who required surgical interruption of pain pathways.

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REFERENCES

1. Müller, L. R.: Studien über den Dermographismus und dessen diagnostische Bedeutung, Deutsch. Z. Nervenheilk. 47:413, 1913.

2. Lewis, T.; Harris, K. E., and Grant, R. T.: Influence of the Cutaneous Nerves on Various Reactions of the Cutaneous Vessels, Heart 14:1, 1927.

3. Lewis, T., and Marvin, H. M.: Observations Relating to Vasodilatation Arising from Antidromic Impulses, to Herpes Zoster and Trophic Effects, Heart 14:27, 1927.

4. Lewis, T.: The Blood Vessels of the Human Skin and Their Responses, London, Shaw & Sons, 1927.

5. Cotton, T. F.; Slade, J. G., and Lewis, T.: Observations Upon Dermatographism, With Special Reference to the Contractile Power of Capillaries, Heart 6:227, 1915-1917.

6. Lewis, T., and Grant, R. T.: Vascular Reactions of the Skin to Injury, The Liberation of a Histamine-Like Substance in Injured Skin; The Underlying Cause of Factitious Urticaria and of Wheals Produced by Burning; Observations upon the Nervous Control of Certain Skin Reactions, Heart 11:209, 1924.

7. Bilisoly, F. N.; Goodell, H., and Wolff, H. G.: Vasodilatation, Lowered Pain Threshold, and Increased Tissue Vulnerability, A.M.A. Arch. Intern. Med. 94:759, 1954.

8. Chapman, L. F.; Goodell, H., and Wolff, H. G.: Neurohumoral Features of the Axon Reflex, Fed. Proc. 18:25, 1959.

9. Graham, D. T.; Goodell, H., and Wolff, H. G.: Studies on Pain: The Relation Between Cutaneous Vasodilatation, Pain Threshold, and Spontaneous Itching and Pain, Amer. J. Med. Sci. 234:420, 1957.

10. Chapman, L, F.; Goodell, H., and Wolff, H. G.: Increased Inflammatory Reaction Induced by Central Nervous System Activity, Trans. Ass. Amer. Physicians 72:84, 1959.

11. Chapman, L. F.; Goodell, H., and Wolff, H. G.: Structures and Processes Relevant to the Sensation of Itch, in The Innervation of the Skin, edited by W. Montagna, New York, Pergamon Press, 1960.

12. Goltz, F.: Uber gefasserweiternde Nerven, Pflueger Arch. Ges. Physiol. 9:185, 1874.

13. Stricker, S.: Untersuchung über die Geffässerwurzeln des Ischiadicus, Sitz. Kaiserl. Akad. Wissenchr. (Wien) 3:173, 1876.

14. Bayliss, W. M.: On the Origin from the Spinal Cord of the Vasodilator Fibers of the Hind Limb, and the Nature of These Fibers, J. Physiol. 26:173, 1901.

15. Bayliss, W. M.: Further Researches on Antidromic Nerve Impulses, J. Physiol. 28:276, 1902.

16. Bayliss, W. M.: The Excitation of Vasodilator Fibers in Depressor Reflexes, J. Physiol. 37:264, 1908.

17. Bayliss, W. M.: The Vasomotor System, London, Longmans, Green & Co., Inc., 1923.

18. Langley, J. N.: Antidromic Action, Part I, J. Physiol. 57:428, 1923.

19. Langley, J. N.: Antidromic Action, Part II. Stimulation of the Peripheral Nerves of the Cat's Hind Foot, J. Physiol. 58:70, 1923.

20. Langley, J. N.: The Autonomic Nervous System, London, Cambridge University Press, 1921.

21. Hinsey, J. C., and Gasser, H. S.: The Component of the Dorsal Root Mediating Vasodilatation and the Sherrington Contracture, Amer. J. Physiol. 92:679, 1930.

22. Gasser, H. S.: Unmedulated Fibers Originating in Dorsal Root Ganglia, J. Gen. Physiol. 33:651, 1950.

23. Gaskell, W. H.: The Involuntary Nervous System, London, Longmans, Green & Co., Inc., 1916.

24. Doupe, J.: Studies in Denervation. D. The Mechanism of Axonal Vasodilatation, J. Neurol. Neurosurg. Psychiat. 6:115, 1953.

25. Kibjakow, A. W.: Zur Frage des Vasodilatationsmechanismus bei der Reizung antidromer Nerven: I. Über die gefässerweiternden Eigenschaften des Blutes beim Reiz der hinteren sensiblen Wurzeln, Pflueger Arch. Ges. Physiol. 228:30, 1931.

26. Foerster, O.: Über die antidrome Leitung sensiblen Nerven., Vol. Jubilaire pour Rossolimo, Moscow, 1925, p. 145.

27. Foerster, O.: Die Leitungsbahnen des Schmerzgefühls und die chirurgische Behandlung des Schmerzziistande, Berlin, Urban & Schwartzenberg, 1927.

28. Krogh, A., Harrop, G. A., and Rehberg, P. B.: Studies on the Physiology of Capillaries, J. Physiol. 56:179, 1922.

29. Krogh, A.: The Anatomy and Physiology of Capillaries, New Haven, Conn., Yale University Press, 1929.

30. Ungar, G.: Effet de l'excitation du bout périphérique des nerfs sensitifis sur la sécrétion gastrique. Transmission neuro-humorale histaminique, C.R. Soc. Biol. (Par.) 118:620, 1935.

31. Ungar, G.: Demonstration de la mise en liberté de substances histaminiques, Transmission neuro-humorale histaminergique, J. Physiol. Path. Gen. 34:77, 1936.

32. Ungar, G.: Les substances histaminiques et la transmission chimique de l'influx nerveux. L'histaminergie normale et pathologique, Paris, Hermann et Cie, 1937.

33. Ungar, G., and Parrot, J. L.: Sur la nature de la substance libérée au cours de la vasodilatation dite antidromique, C.R. Soc. Biol. (Par.) 129:753, 1938.

34. Ungar, G., and Parrot, J. L.: Sur les rapports entre la substance libérée au cours de la vasodilatation antidromique et le corps hypotenseur produit par la transformation enzymatique de l'adrénaline, C.R. Soc. Biol. (Par.) 131:1165, 1939.

35. Hara, K.: Über die Hemmung der Schweissekretion nach Reizung der hinteren Wurzel, zugleich als ein Beitrag über die Wirkungsweise der sogenannten antidromen Nervenerregung, Pflueger Arch. Ges. Physiol. 221:692, 1928.

36. Kwiatkowski, H.: Histamine in Nervous Tissue, J. Physiol. 102:32, 1943.

37. Ibrahim, F. O.; Stella, G., and Talaat, M.: The Mechanism of Antidromic Vasodilatation, Quart. J. Exp. Physiol. 36:189, 1951.

38. Parrot, J. L., and Lefcbvre, J.: Analyse de la triple réaction cutanée au moyen d'un antagoniste de l'histamine, C.R. Soc. Biol. (Par.) 137:316, 1943.

39. Holton, P., and Perry, W. L. M.: On the Transmitter Responsible for Antidromic Vasodilatation in the Rabbit's Ear, J. Physiol. 114:240, 1951.

40. Frumin, N. J.; Ngai, S. H., and Wang, S. C.: Evaluation of Vasodilatator Mechanisms in the Canine Hind Leg: Question of Dorsal Root Participation, Amer. J. Physiol. 173:428, 1953.

41. Ungar, G.: Sur les rapports des appareils périphériques vasodilatateurs avec les terminasons nerveuses sensitives, d'après la conception de la transmissicm humorale histaminique, Ann. Anat. Path. 12:586, 1935.

42. von Euler, U. S.: Sympathin Histamine and Acetylcholine in Mammalian Nerves, J. Physiol. 107:106, 1948.

43. von Euler, U. S.: Histamine as a Specific Constituent of Certain Autonomic Nerve Fibers, Acta Physiol. Scand. 19:85, 1949.

44. Werle, E., and Weichen, G.: Uber das Vorkommen von Histamin in Nerven, Biochem. Z. 319:457, 1949.

45. Werle, E., and Palm, D.: Histamin in Nerven, II, Biochem. Z. 320:322, 1950.

46. von Euler, U. S., and Åström, A.: Liberation of Histamine and Sympathin by Stimulation of Isolated Splenic Nerves of Cattle, Acta Physiol. Scand. 16:97, 1949.

47. Dale, H. H.: Chemical Transmission of the Effect of Nerve Impulses, Brit. Med. J. 1:835, 1934.

48. Dale, H. H., and Gaddum, J. H.: Reactions of Denervated Voluntary Muscle, and Their Bearing on the Mode of Action of Parasympathetic and Related Nerves, J. Physiol. 70:109, 1930.

49. Phillipeaux, J. M., and Vulpian, E. F. A.: Note sur une modification physiologique qui se produit dans le nerf lingual par suite de l'abolition temporaire de la motricité—dans le nerf hypoglosse du méme cote, C.R. Acad. Sci. (Par.) 56:1009, 1863.

50. Phillipeaux, J. M., and Vulpian, E. F. A.: Nouvelles experiences sur l'existence de fibres nerveuses vasodilatrices dans les racines posterieures des 4 et 5 nerfs lombaires chez le chien, Arch. Physiol. 5:336, 1878.

51. Hinsey, J. D., and Cutting, C. C.: The Sherrington Phenomenon. V. Nervous Pathways, Amer. J. Physiol. 105:535, 1933.

52. Bulbring, E., and Burn, J. H.: The Sympathetic Dilator Fibers in the Muscles of the Cat and Dog, J. Physiol. 83:483, 1935.

53. Fleisch, A., and Cavin-Franel. L.: Une substance intermédiaire de la vasodilatation, Arch. Int. Physiol. 42:516, 1936.

54. Wybauw, L.: Transmission humorale de la vasodilatation provoquée par l'excitation du bout périphérique des racines postérieures lombaires chez le chat, C.R. Soc. Biol. (Par.) 123:524, 1936.

55. Wybauw, L.: Contribution a l'étude du rôle vasomoteur et trophique des nerfs sensitifs, Arch. Int. Physiol. 46:293, 1938.

56. Folkow, B., and Uvnas, B.: Do Adrenergic Vasodilator Nerves Exist? Acta. Physiol. Scand. 20:329, 1950.

57. Bernard, C. L.: Sur le variations de coleur dans le sang veineux des organes glandulaire, J. Physiol. l'Homme Animaux 1:233, 1858.

58. Heidenhain, R.: Über die Wirkung einiger Gifte auf die Netven der Glandula submaxillaris, Pflueger Arch. Ges. Physiol. 5:309, 1872.

59. Babkin, B. P.; Gibbs, O. S., and Wolff, H. G.: Die humorale Übertragung des chorda tympani-Reizung, Arch. Exp. Path. Pharmakol. 168:32, 1932.

60. Ungar, G.; Parrot, J. L., and Grossiord, A.: Stimulation de la sécrétion gastrique par l'excitation du bout périphérique du nerf lingual. Passage d'une substance active dans la salive, C.R. Soc. Biol. (Par.) 121:1077, 1936.

61. Feldberg, W., and Guimarais, J. A.: Some Observations on Salivary Secretion, J. Physiol. 81:65, 1934.

62. Ungar, G., and Parrot, J. L.: Sur la présence de la callicréine dans la saliva et la possibilité de son intervention dans la transmission chimique de l'influx nerveux, C.R. Sac. Biol. (Par.) 122:1052, 1936.

63. Frey, E. K.: Zusammenhänge zwischen Herzarbeit und Nierentatigkeit, Arch. Klin. Chir. 142:663, 1925.

64. Werle, E.; Gotze, W., and Keppler, A.: Über die Wirkung des Kallikreins auf den isoletieren Darm und über eine neue darmkontrahierende Substanz, Biochem. Z. 289:217, 1937.

65. Frey, E. K., and Kraut, H.: Ein neues Kreizlaufhormon und seine Wirkung, Arch. Exp. Path. Pharmacol. 133:1, 1928.

66. Frey, E. K.; Kraut, H., and Werle, E.: Kallikrein; Padutin, Stuttgart, Ferdinand Enke, 1950.

67. Szakall, A.: Morphologische Veranderungen nach intervenoser Kallikreinzufuhr als Ursache der Wirkungen auf den Stoffwechsel, Biochem. Z. 269:92, 1934.

68. Hilton, S. M., and Lewis, G. P.: The Cause of the Vasodilatation Accompanying Activity in the Submandibular Salivary Gland, J. Physiol. 128:235, 1955.

69. Hilton, S. M., and Lewis, G. P.: The Mechanism of Functional Hyperemia in the Submandibular Salivary Gland, J. Physiol. 129:253, 1955.

70. Hiltcm, S. M., and Lewis, G. P.: The Relationship Between Glandular Activity, Bradykinin Formation, and Functional Vasodilatation in the Submandibular Salivary Gland, J. Physiol. 134:471, 1956.

71. Fox, R. H., and Hiltcm, S. M.: Bradykinin Formation in Human Skin as a Factor in Heat Vasodilatation, J. Physiol. 142:219, 1958.

72. Hilton, S. M., and Lewis, G. P.: Vasodilatation in the Tongue and Its Relationship to Plasma Kinin Formation, J. Physiol, 144:532, 1958.

73. Hellauer, H. F., and Umrath, K.: Uber die Aktionsubstanz der sensiblen Nerven, Pflueger Arch. Ges. Physiol. 249:619, 1948.

74. Lembeck, F.: Zur Frage der zentralen Übertragung afferenter Impulse, III, Arch. Exp. Pharmakol. 219:197, 1953.

75. von Euler, U. S., and Gaddum, J. H.: An Unidentified Depressor Substance in Certain Tissue Extracts, J. Physiol. 72:74, 1931.

76. Gaddum, J. H., and Schild, H.: Depressor Substances in Extracts of Intestine, J. Physiol. 83:1, 1934.

77. Gaddum, J. H., Editor: Polypeptides Which Stimulate Smooth Muscle, Edinburgh, E. & S. Livingstone, Ltd., 1955.

78. Schachter, M., Editor: Polypeptides Which Affect Smooth Muscles and Blood Vessels, New York, Pergamon Press, 1960.

79. Pernow, B.: Studies on Substance P Purification, Occurrence, and Biological Actions, Acta. Physiol. Scand. (Suppl. 105) 29:1, 1953.

80. Zetler, G., and Schlosser, L.: Uber die Verteilung von Substanz P und Cholinacetylase im Gehirn, Arch. Exp. Path. Pharmakol. 224: 159, 1955.

81. Kopera, H., and Lazarini, W.: Zur Frage der zentralen Übertragung afferenter Impulse, IV, Arch. Exp. Path. Pharmakol. 219:214, 1953.

82. Amin, A. H.; Crawford, T. B. B., and Gaddum, J. H.: The Distribution of Substance P and 5-Hydraxytryptarnine in the Central Nervous System of the Dog, J. Physiol. 126:596, 1954.

83. Andrews, T. M., and Holton, P.: The Substance P and Adenosine Triphosphate (ATP) Contents of Sensory Nerves on Degeneration, J. Physiol. 143:45P, 1958.

84. Holton, P.: Substance P Concentration in Degenerating Nerve.*

85. Holton, F. A., and Holton, P.: The Possibility that ATP is a Transmitter at Sensory Nerve Endings, J. Physiol. 119:50P, 1953.

86. Holton, P.: The Liberation of Adenosine Triphosphate ***on*** Antidromic Stimulation of Sensory Nerves, J. Physiol. 145:494, 1959.

87. Strehler, 3. L., and Totter, J. R.: Determination of ATP and Related Compounds, Meth. Biochem. Anal. 1:341, 1954.

88. Brown, G. L.; Dale, H. H., and Feldberg, W.: Reactions of the Normal Mammalian Muscle to Acetylcholine and to Eserine, J. Physiol. 87: 394, 1936.

89. Breslauer, F.: Die Pathogenese der trophischen Gewebeschaden nach der Nervenverletzung, Deutsch. Z. Chir. 150:50, 1919.

90. Ebbeke, U.: Die lokale vasomotorische Reaktion (L.VR) der Haut und der inneren Organe, Pflueger Arch. Ges. Physiol. 169:1, 1917.

91. Bruce, A. N.: Uber die Berziehung der sensiblen Nervendigungen zum Entzundungsvorgang, Arch. Exp. Path. Pharmakol. 63:424, 1910.

92. von Bardy, H.: Uber Hemmung inflammatorischer Symptome, Skand. Arch. Physiol. 32: 198, 1915.

93. Langley, J. N., and Anderson, H. K.: On Reflex Action from Sympathetic Ganglia, J. Physiol. 16:410, 1894.

94. Woollard, H.: The Innervation of Blood Vessels, Heart 13:319, 1926.

95. Weddell, G.: The Anatomy of Cutaneous Sensibility, Brit. Med. Bull. 3:167, 1945.

96. Weddell, G.; Sinclair, D. C., and Feindel, W. H.: An Anatomical Basis far Alteration in the Quality of Pain Sensibility, J. Neurophysiol. 11:99, 1948.

97. Adrian, E. D.; Cattell, McK., and Hoagland, H.: Sensory Discharges in Single Cutaneous Nerve Fibers, J. Physiol. 72:392, 1931.

98. Celander, O., and Folkow, B.: The Nature and the Distribution of Afferent Fibers Provided with the Axon Reflex Arrangement, Acta Physiol. Scand. 29:359, 1953.

99. Celander, O., and Folkow, B.: The Correlation Between the Stimulation Frequency and the Dilator Response Evoked by "Antidromic" Excitation of the Thin Afferent Fibers in the Dorsal Roots, Acta Physiol. Scand. 29:371, 1953.

100. Parrot, J. L.: Hypothese sur la nature des fibres nerveuses qui interviennent dans le reflexe d'axone vasodilateur, Comm. Soc. Anat., (Par.) 1:3, 1943.

101. Parrot, J. L.: The Place of Histamine in Neurohumoral Transmission, Pharmacol. Rev. 6: 110, 1954.

102. Kure, K.: Uber den Spinalparasympathikus, Basel, Schwabe, 1931.

103. Duncan, D., and Keyser, L. L.: Further Determination of the Numbers of Fibers and Cells in the Dorsal Roots and Ganglia of the Cat, J. Comp. Neurol. 68:479, 1938.

104. Parrot, J. L.: Les modifications apportées par un antagoniste de l'histamine (2339 RP) à la réaction vasculaire locale de la peau, C.R. Soc. Biol. (Par.) 136:715, 1942.

105. Ostield, A.; Chapman, L. F.; Goodell, H., and Walff, H. G.: A Summary of Evidence Concerning a Noxious Agent Active Locally During Migraine Headache, Psychosom. Med. 19:199, 1957.

106. Zileli, T.: Unpublished observation.

107. Habgood, J. S.: Sensitization of Sensory Receptors in the Frog's Skin, J. Physiol. III:195, 1950.

108. Rocha e Silva, M.; Beraldo, W. T., and Rosenfeld, G.: Bradykinin, a Hypotensive and Smwth Muscle Stimulating Factor Released from Plasma Globulin by Snake Venom and by Trypsin, Amer. J. Physiol. 156:261, 1949.

109. Lewis, G. P.: Active Polypeptides Derived from Plasma Proteins, Physiol. Rev. 40:647, 1960.

110. Chapman, L. F., and Wolff, H. G.: A Property of Cerebrospinal Fluid Indicating Disturbed Metabolism of the Central Nervous System, Science 128:1208, 1958.

111. Chapman, L. F., and Wolff, H. G.: Studies of Proteolytic Enzymes in cerebrospinal Fluid, A.M.A. Arch. Intern. Med. 103:86, 1959.

112. Chapman, L. F.; Ramos, A. O.; Corrado, A.; Fortes, V., and Symmes, D.: Release of Proteolytic Enzyme by Stimulation of Cat Brain, A.M.A. Arch. Neurol. 3:43, 1960.

113. Konzett, H.: Pharmacology of Oxytocin, Vasopressin, and Analogoues.[78]

114. Rocha e Silva, M.: Biochemistry and Pharmacology of Bradykinin.[78]

115. Werle, E.: Kallikrein, Kallidin, and Related Substances."

116. Sherrington, C. S.: On the Anatomical Constitution of Nerves of Skeletal Muscles, with Remarks on Recurrent Fibres in the Ventral Spinal Nerve-Root, J. Physiol. 17:217, 1894.

117. Benjamin, F. B.: Relationship Between Injury and Pain in the Human Skin, J. Appl. Physiol. 5:740, 1953.

118. Gotch, F., and Horsely, V.: On the Mammalian Nervous System, Its Functions and Their Localization, Determined by an Electrical Method, Philos. Trans. 182:267, 1891.

119. Barron, D. H., and Mathews, B. H.: "Recurrent Fibres" of the Dorsal Roots, J. Physiol. 85:104, 1935.

120. Toennies, J. F.: Reflex Discharge from the Spinal Cord Over the Dorsal Roots, J. Neurophysiol. 1:378, 1938.

121. Toennies, J. F.: Conditioning of Afferent Impulses by Reflex Discharges Over the Dorsal Roots, J. Neurophysiol. 2:515, 1939.

122. Wall, P. D.: Excitability Changes in Afferent Fibre Terminations and Their Relation to Slow Potentials, J. Physiol. 142:1, 1958.

123. McCouch, G. P., and Austin, G. M.: Postsynaptic Source of Dorsal Root Reflex, J. Neurophysiol. 21:217, 1958.

124. Tregear, R. T.: The Relation of Antidromic Impulses in the Dorsal Root Fibers to the Dorsal Root Potential in the Frog, J. Physiol. 142:343, 1958.

125. Habgood, J. S.: Antidromic Impulses in the Dorsal Roots, J. Physiol. 121:264, 1953.

126. Ansell, G. B., and Richter, D.: Evidence far a Neutral Proteinase in Brain Tissue, Biochim. Biophys. Acta 13:92, 1954.

127. Geiger, A.; Dobkin, J., and Magnus, J.: Accumulation of Acid-Soluble Nitrogen in the Brain Cortex of Cats During stimulation, Science, 118:655, 1953.

128. Ungar, G.; Ascheim, E.; Psychoyos, S., and Romano, D. V.: Reversible Changes of Protein Configuration in Stimulated Nerve Structures, J. Gen. Physiol. 40:635, 1956.

129. Ungar, G., and Romano, D. V.: Sodium "Binding" and Protein Structure in Stimulated Rat Brain, Fed. Proc. 18:162, 1959.

130. Elliott, D. F.; Lewis, G. P., and Horton, E. W.: The Isolation of Bradykinin: A Plasma Kinin from Ox Blood, Biochem. J. 74:15p, 1959.

131. Boissonas, R. A.; St. Guttman, P. A.; Quenoud, J. A.; Konzett, H., and Stürmer, E.: Synthesis and Biological Activity of Peptides Related to Bradykinin, Experientia 16:326, 1960.

132. Fox, R. H.; Goldsmith, R.; Kidd, D. J., and Lewis, G. P.: Bradykinin as a Vasodilator in Man, Proc. Physiol. Soc., July, 1960, pp. 29-30.

133. Penfield, W.; von Santha, K., and Cipriani, A.: Cerebral Blood Flow During Induced Epileptiform Seizures in Animals and Man, J. Neurophysiol. 2:257, 1939,

134. Ingvar, D. H.: Cortical State of Excitability and Cortical Circulation, in The Reticular Formation of the Brain, Boston, Little, Brown & Company, 1958, Ch. 19.

135. Chapman, L. F., and Ramos, A. O.: Unpublished observations.

136. Dale, H. H.: Reizübertragung durch chemische Mittell im peripheren Nervensystem (Nothnagelstiftung Vortrag), Vienna, Urban and Schwarzenberg, 1935.

137. Feldberg, W. S.: Transmission in the Central Nervous System and Sensory Transmission: Central and Sensory Transmission, Pharmacol. Rev. 6:85, 1954.

138. Häusler, H. F., and Stertz, H.: Zur Frage der Ubertragung sensibler Impulse im Riickenmark des Frosches, J. Mount Sinai Hospital N.Y. 19:121, 1952.

139. Uvnäs, B.: Antidromic Vasodilatation in the Paw of the Cat, Pharmacol. Rev. 6:99, 1954.

140. Collier, J., and Adie, W. J.: Tabes Dorsalis, in Textbook of the Practice of Medicine, Ed. 4, edited by Frederick W. Price, London, Oxford University Press, 1934, Sect. 20, p. 1593.

141. Reilly, J.; Rivalier, E.; Compagnon, A.; Lapane, R., and Du Buit, H.: Sur le pathogenie de la dothiénentérie. La fièvre typhoide experimentale. Le rôle du systeme nerveux végétatif dans le genèse des lesion intestinales, Ann. Med. 37:182, 322, 1935.

142. Tinel, J.; Ungar, G.; Grossiord, A., and Parrot, J. L.: Récherches sur la production experi-

mentale de lesions abdominales par l'excitation des racines posterieures, Ann. Anat. Path. 14:213, 1937.

143. Carton, C. A., and Kilbourne, E. D.: Activation of Latent Herpes Simplex by Trigeminal Sensory Root Section, New Engl. J. Med. 246: 172, 1952.

144. Carton, C. A.: Effect of Previous Sensory Loss on the Appearance of Herpes Simplex Following Trigeminal Sensory Root Section, Trans. Amer. Neurol. Ass. 1952, p. 154.

145. Sweet, W. H.: Discussion of Carton's paper (reference 144).

146. Chapman, L. F.; Ramos, A. O.; Goodell, H.; Silverman, G., and Wolff, H. G.: A Humoral Agent Implicated in Vascular Headache of the Migraine Type, A.M.A. Arch. Neurol. 3:223, 1960.

147. Chapman, L. F.; Goodell, H., and Wolff, H. G.: Changes in Tissue Vulnerability Induced During Hypnotic Suggestion, J. Psychosom. Res. 4:99, 1959.

148. Lewis, T.: Pain, New York, The Macmillan Company, 1942.

149. Hardy, J. D.; Wolff, H. G., and Goodell, H.: Pain Sensations and Reactions, Baltimore, Williams and Wilkins Company, 1952.

150. Moritz, A. R., and Henriques, F. C.: Studies of Thermal Injury. II. The Relative Importance of Time and Surface Temperature in the Causation of Cutaneous Burns, Amer. J. Pathol. 23:695, 1947.

151. Rosenthal, S. R., and Minard, D.: Experiments on Histamine as the Chemical Mediator for Cutaneous Pain, J. Exper. Med. 70:415, 1939.

152. Parrot, J. L.: Sur le mécanisme périphérique de la douleur. Intervention de l'histamine dans la brûlure et le prurit, C.R. Soc. Biol. (Par.) 137:620, 1943.

153. Rosenthal, S. R.: Histamine as Possible Chemical Mediator for Cutaneous Pain. Dual Pain Response to Histamine, Proc. Soc. Exp. Biol. Med. 74:167, 1950.

154. Ambrus, J. L., and Ambrus, C. M.: The Role of Histamine in the Function of Sensory Nerves, Z. Vitamin Hormone Fermentforsch. 5: 45, 1952.

155. Riley, J. F., and West, G. B.: The Presence of Histamine in Tissue Mast Cells, J. Physiol. 120:528, 1953.

156. Menkin, V.: Studies on Inflammation. XVI. Formation of a Chemotactic Substance by Enzymatic Action, J. Exp. Med. 67:153, 1938.

157. Day, T. D.: Localizing Action of Fibrinous Exudates, Brit. J. Exp. Pathol. 35:315, 1954.

158. Duthie, E. S., and Chain, E.: A Polypeptide Responsible for Some of the Phenomena of Acute Inflammations, Brit. J. Exp. Pathol. 20:417, 1939.

159. Culumbine, H., and Rydon, H. N.: A Study of the Formation, Properties and Partial Purification of Leukotaxine, Brit. J. Exp. Pathol. 27:33, 1946.

160. Spector, W. G.: Role of Some Higher Peptides in Inflammation, J. Path. Bact. 69:93, 2951.

161. Armstrong, D.; Dry, R. M. L.; Keele, C. A., and Markam, J. W.: Observations on Chemical Excitants of Cutaneous Pain in Man, J. Physiol. 120:326, 1953.

162. Miles, A. A., and Wilhelm, D. L.: Enzyme-like Globulins from Serum Reproducing the Vascular Phenomena of Inflammation. I. An Activable Permeability Factor and Its Inhibitor in Guinea Pig Serum, Brit. J. Exp. Path. 36:71, 1955.

163. Wilhelm, D. L.; Miles, A. A., and Mackay, M. E.: Enzyme-like Globulins from Serum Reproducing the Vascular Phenomena of Inflammaticm. II. Isolation and Properties of the Permeability Factor and Its Inhibitor, Brit. J. Exp. Path. 36:8, 1955.

164. Beloff, A., and Peters, R. A.: Observations Upon Thermal Burns: The Influence of Moderate Temperature Burns Upon a Proteinase of Skin, J. Physiol. 103:461, 1945.

165. Ungar, G., and Hayashi, H.: Enzymatic Mechanisms in Allergy, Ann. Allergy 16:542, 1958.

166. Rocha e Silva, M.: A Comprehensive Theory on the Mechanism of Histamine Release, An. Bras. Acad. Science 31:77, 1959.

167. Bennet, D. W., and Drury, A. N.: Further Observations Relating to the Physiological Activity of Adenine Compounds, J. Physiol. 72:288, 1931.

168. Florey, H. W.: Inflammation: Macroscopial Observations, in Lectures on General Pathology, edited by H. W. Florey, Philadelphia, W. B. Saunders Company, 1954.

169. Armstrong, D.; Keele, C. A.; Jepson, J. B., and Stewart, J. W.: Pain-Producing Substance in Human Inflammatory Exudates and Plasma, J. Physiol. 135:350, 1937.

170. Keele, C. A.: Formation of Pain Producing Polypeptide (PPS) from Plasma.[78]

171. Menkin, V.: Biochemical Mechanisms in Inflammation, Springfield, Charles C Thomas, Publisher, 1956.

172. Benjamin, F. B.: Release of Intracellular Potassium as the Physiological Stimulus for Pain, Fed. Proc. 18:10, 1959.

173. Rocha e Silva, M., and Antonio, A.: Release of Bradykinin in the Area of Edema Produced by Heating the Rat's Paw (at 44-45 C), Pharmacologist 2:89, 1960.

174. Herxheimer, A., and Schachter, M.: Wheal and Flare in Human Skin Produced by Histamine and Other Substances, J. Physiol. 143:34P, 1959.

175. Hardy, J. D.; Wolff, H. G., and Goodell, H.: Experimental Evidence on the Nature of Cutaneous Hyperalgesia, J. Clin. Invest: 29:115, 1950.

176. Lewis, T., and Kellgren, J. H.: Observations Relating to Referred Pain, Visceromotor Reflexes, and Other Associated Phenomena, Clin. Sci. 4:47, 1939.

177. Weddell, G.; Sinclair, D. C.; Feindel, W. H., and Falconer, M. A.: The Intervertebral Ligaments as a Source of Segmental Pain, J. Bone Joint Surg. 30B:515, 1948.

178. McAuliffe, G. W.; Goodell, H., and Wolff, H. G.: Experimental Studies on Headache: Pain from the Nasal and Paranasal Structures, Proc. Ass. Res. Nerv. Ment. Dis. 23:185, 1943.

179. Woollard, H. H.; Roberts, J. E. H., and Carmichael, E. A.: An Inquiry into Referred Pain, Lancet, 1:37, 1932.

180. Lewis, T.: Experiments Relating to Cutaneous Hyperalgesia and Its Spread Through Somatic Nerves, Clin. Sci. 2:373, 1936.

181. McLellan, A., and Goodell, H.: Pain from the Bladder, Ureter, and Kidney Pelvis, Proc. Ass. Res. Nerv. Ment. Dis. 23:252, 1943.

182. Robertson, S.; Goodell, H., and Wolff, H. G.: Headache: The Teeth as a Source of Headache and Other Head Pain, A.M.A. Arch. Neurol. Psychiat. 57:277, 1947.

183. Hardy, J. D.; Goodell, H., and Wolff, H. G.: Studies on Pain: Observations on the Hyperalgesia Associated with Referred Pain, Amer. J. Physiol. 133:316, 1941.

184. Sinclair, D. S.; Weddell, G., and Feindel, W. H.: Referred Pain and Associated Phenomena, Brain 71:184, 1948.

185. Miles, A. A., and Wilhelm, D. L.: Globulins Affecting Capillary Permeability.[78]

186. Gross, F., and Turrian, H.: Pharmacology of Hypertensin and Synthetic Analogues.[78]

187. Croxatto, H.: Active Polypeptides Formed by Pepsin.[77]

188. Gaddum, J. H., and Horton, E. W.: The Extraction of Human Urinary Kinin (Substance Z) and Its Relation to the Plasma Kinins, Brit. J. Pharmacol. Chernother. 14:117, 1959.

189. Ramos, A. O., and Corrado, A. P.: Unpublished observations.

190. Horton, E. W.: Urinary Kinin.[78]

THE "POST HOC" FALLACY

In everyday affairs, and to a certain extent in the general run of clinical work, the number of accepted causal relationships far exceeds the number of demonstrated and even of probable cases. It seems likely, then, that there is some strong natural tendency which inclines us to ascribe causal relations to events accidentally and arbitrarily occurring near one another. That we have such a tendency is at least suggested by the work of Pavlov on conditioned reflexes. The essential facts of this work are familiar and need not be recalled in detail. In a dog, in which a salivary fistula has been established so that the flow of saliva can be measured, the presentation of food is followed at once by a profuse secretion. Some arbitrarily chosen stimulus such as the sounding of a metronome is now made to precede immediately the offer of food. After this sequence has been repeated a few times it is found that the auditory stimulus causes a similar salivary flow without any presentation of food. The cerebral cortex has been taught by a very short experience to accept the metronome sound as a precursor of food. In mental terms the sound has become a 'cause' of food, and an intelligent dog might classify metronomes in the group of things to be hunted like rats and rabbits. The facts especially to be noted for our purpose here are: first, the readiness with which the link of apparent causation has been formed; secondly, the arbitrariness of the sequence, metronomes having no relation with food in the general objective world, and thirdly, the fact that the relation can be fixed only by adding it to a relation already established—namely, a natural reflex—in this case the visual-salivary reflex.

Enough has been said . . . to enjoin caution in applying the results of experiments in animals to man. Caution must, moreover, be especially stringent in seeking light on mental problems from cerebral phenomena. Nevertheless, it is clear enough that there is a remarkably close analogy between the aptitude for the formation of conditioned reflexes on the one hand and on the other hand the tendency too readily to ascribe the causal relation to small and unrelated sequences of events; and we may at least suspect that the subjective sense of causation we so easily derive from a few random coincidences is connected with the proved capacity of the brain to pick up arbitrary and as it were 'irrational' reflexes. The conditioned reflex then is perhaps the simplest case of the post hoc fallacy. When we thus see reason to suspect that our tendency to impute causal relations unjustifiably may be the expression of a characteristic cerebral function we need not be surprised that this fallacy is so common and so difficult to avoid.—Observations and Experiment and Their Use in the Medical Sciences, The Collected Papers of Wilfred Trotter, F.R.S., Oxford University Press, Oxford and New York, 1943.